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Hae Koo KIM

TITRE :

MODELLING ENVIRONMENTAL AND GENETIC CONTROL OF TILLERING IN SORGHUM

MODELISATION DU CONTROLE ENVIRONMENTAL ET GENETIQUE DU TALLAGE CHEZ LE SORGHO

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Jérémie LECOEUR, Professeur de SUPAGRO	Président
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Gilles LEMAIRE, Directeur de Recherche, INRA de Lusignan	Rapporteur
Claude WELCKER, Ingénieur de Recherche au LEPSE, INRA de Montpellier	Examinateur

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From a worldwide travelling nomad
Dreaming of 'Green Revolution' in Africa, my land of adoption
For its People…

In memory of my Grand-Father,

KIM Kyung-Rok

김 경 록

(1921-2006)

MODELLING GENETIC & ENVIRONMENTAL CONTROL OF TILLERING IN SORGHUM

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"Consider how the lilies grow.
They do not labor or spin.
Yet I tell you,
not even Solomon in all his splendor
was dressed like one of these.

If that is how God clothes the grass of the field,
which is here today,
and tomorrow is thrown into the fire,
how much more will he clothe you,
O you of little faith!

And do not set you heart on
what you will eat or drink;
do not worry about it. "

CHAPTER I

GENERAL INTRODUCTION
AND STATE OF THE ART

Importance of tillering for grain yield in cereals

Crop cultivars with higher yield potential are the key to increase productivity (Evans, 1993). This necessity is today reinforced by the worldwide food crisis in order to feed a world population expected to exceed 9 billion people by 2050 (Alexandratos, 1999; Gilland, 2002), as highlighted at the 5th International Crop Science Congress (Nelson, 2008). Cereal productivity plays a major role in this context (FAO statistics, 2006). Yield formation in cereals is a complex but coordinated process that integrates numerous components involved during vegetative and reproductive growth phases, including tillering (panicle number), panicle size (spikelet number per panicle), fraction of fertile spikelets, and grain weight (filling) (Evans and Wardlaw, 1976; Evans, 1993). Also, variations in crop growth and development throughout the crop cycle, due to genotype by environment interactions (GxE), can strongly affect final grain yield. A better understanding of how a given yield component is controlled by GxE is thus essential to improve plant adaptive capacity and hence cereal crop productivity.

One of the approaches that led to a large increase in yield potential of cereals was the modification of 'plant type' (Donald, 1968). For instance, to increase the genetic yield potential of the tall, conventional rice plant type, a modern rice type was bred for semi-dwarf, high tillering behaviour. Based on this experience and to attempt to further raise rice harvest index, a new plant type (NPT) was conceptualized (Yang *et al.*, 2007), consisting of a modified architecture, in particular a reduction of tiller number, an increase of the number of grains per panicle and straw stiffness (Khush, 1999). The ideotype proposed by Donald (1968) in wheat was low tillering to reduce plant-plant competition and reduce wasteful use of limited resources when grown in a monoculture situation in particular in water-limited situations. In either cases, tillering regulation represents a key morphogenetic yield component of grain cereals (e.g. rice, wheat, barley, millet, sorghum), producing either a large

number of small panicles or a reduced number of larger panicles per unit area.

Tillering rate and phase duration differ greatly among cereal species. Tiller number can range from none (monoculm crops such as modern maize) to up to hundreds per square meter under field conditions. The potential tillering ability of some grasses (barley, wheat, rice) even under unfavourable conditions remains superior to sparsely tillering species (sorghum, maize) grown in optimal environments. However, not all tillers produced are necessarily fertile, and the contribution of tillering to grain yield thus depends strongly on environment, particularly competition for resources within the population (Lafarge *et al.*, 2002). Thus, both outgrowth and fertility of tillers have to be considered to increase yield potential (Sakamoto and Matsuoka, 2004), particularly in medium tillering cereals whose grain yield is significantly influenced by tiller number (Hart *et al.*, 2001; Lafarge and Hammer, 2002a).

Recent investigations on architectural development in grasses illustrated that branching is a crucial trait for cereal domestication, based on a change in the pattern and timing of branching, affecting both vegetative and inflorescence architecture, and ultimately yield (Doust, 2007a). Today, cereal grasses exhibit two main forms of vegetative architecture: the pooid and erhartoid cereals such as wheat and rice have multiple basal tillers, while panicoid cereals such as maize and sorghum mostly have few tillers or even only a single main stem. A combination of phylogenetic and genomic analysis is beginning to reveal the similarities and differences between different cereal crops, and to relate them to the diversity of wild ancestors (Doust, 2007b). Recent advances in plant genomics and studies on genes controlling branching emphasize the necessity of an inter-disciplinary approach integrating plant physiology, modelling and genetics to close the gap between gene function and plant phenotype and thus to enhance plant breeding. Complex traits need to be examined in a wider framework in order to detect morphology adapted to agro-ecological environments (Cooper *et al.*, 2002b; Cooper *et al.*, 2005; Whish *et al.*, 2005). Consequently, branching has been a

decisive trait for both natural selection and breeding of many species, particularly cereals. It is still today a key component trait for increasing yield, but is a complex trait prone to GxE and as such not fully understood and difficult to study in prolifically tillering species.

Sorghum as a complementary cereal model plant

The *Poaceae* family, commonly called grasses, is a monophyletic family of monocotyledonous flowering plants with more than 10,000 species (Clark *et al.*, 1995), including the cereal crops rice, maize, wheat, barley, and sorghum. The haploid nuclear genome size varies considerably among cereals from 450 Mb in rice to 5,000 Mb in barley (Bennett and Leitch, 1995). However, comparative genetic maps of closely related species like sorghum and maize (Hulbert *et al.*, 1990), or of more distantly related cereals, revealed significant genome conservation, despite differences in nuclear DNA content. The discovery of conserved DNA sequences and order (i.e., colinearity) in grasses has not only opened new frontiers for gene (and gene function) discovery, but has also revealed the importance of the study of simple model plants to understand more complex species.

With one of the smallest genomes in the plant kingdom (115Mb), Arabidopsis thaliana has proved to be an ideal model plant for studying plant development among the angiosperms, especially for dicotyledonous plants, despite being an insignificant weed with no commercial importance. Rice (*Oryza sativa* L.) has emerged as the model plant for monocotyledonous crops, mainly because of its small genome (smallest among cereals) and its exceptional agricultural importance (Izawa and Shimamoto, 1996). The full genome sequence was determined by a common worldwide effort led by the International Rice Genome Sequencing Project (Sasaki and Burr, 2000; Sasaki et al., 2002). Sorghum has also just been fully sequenced (Bowers *et al.*, 2007; Kresovich *et al.*, 2005). Rice researchers have developed a comprehensive array of physiological, molecular, genetic, and genomic tools that allow the

precise characterization of rice genome organization and gene function (Bennetzen and Ma, 2003). Studies of the organization of grass genomes indicated that individual rice chromosomes were highly colinear with those of important cereals like maize, barley, wheat and other grass species (Ahn *et al.*, 1993; Ahn and Tanksley, 1993; Moore *et al.*, 1995). These studies led to the prediction that grasses could be studied as a single syntenic genome (Bennetzen and Freeling, 1997). The unified grass genome model had a profound impact on plant biology, but to date the effective outcomes of synteny and colinearity among cereals have not matched initial expectations (Freeling, 2001). The extrapolation of information from the model species to less studied crops, which include many tropical species, was to provide a new basis for their improvement (Gale and Devos, 1998). But up to now, colinearity proved good enough for map-based cloning only in the small-genome model species, Arabidopsis and rice.

Two major reasons might explain the relatively slow application of this approach. First, full genome sequences for other grass species are not yet available (maize and even wheat genome sequencing projects are underway (Chandler and Brendel, 2002; Pennisi, 2007). Second, colinearity of gene order and content observed at the recombinant map level is often not observed at the level of local genome structure (Bennetzen and Ramakrishna, 2002; Feuillet and Keller, 2002). As for *Arabidopsis thaliana* in the case of dicotyledons, the relatively simple genome of rice does not provide full insight into more complex genomes, like the probable tetraploid genome of maize (Ilic *et al.*, 2003), allohexaploid genome of wheat (Moore *et al.*, 1995) or polyploid genome of sugarcane (Ming *et al.*, 1998). Thus, researchers realized that full sequencing of other intermediate genome size species would be necessary to understand more complex genomes of high economic and agronomic importance like maize, wheat or barley, as it would require tremendous effort to fully sequence them directly. Apart from its increasing economic and agro-environmental importance (FAO statistics, 2006; Gnansounou *et al.*, 2005; Tuck *et al.*, 2006), the relatively small sorghum

genome (750Mb) can serve as a complementary reference genome for the much larger and more complex genome of maize (2,400Mb). The sorghum genome was completely sequenced in 2007 and it is expected to provide another powerful resource for comparative genomics among the grasses in particular, and across the plant kingdom in general (Bedell *et al.*, 2005). Analysis of structural and functional genomic similarities including orthologs will strengthen the knowledge gained through *Arabidopsis* and *Oryza sativa* (Ilic *et al.*, 2003) but it is also expected to benefit closely related species like maize and sugarcane (Ming *et al.*, 1998).

As a model organism for tropical grasses with C_4 photosynthesis pathway, sorghum is a complementary model to rice (the first monocot plant fully sequenced), which has C_3 photosynthesis. The genome of sorghum is less complex to assemble than the larger and more repetitive genomes of other major C_4 crops (such as maize or sugarcane). Sequencing of sorghum is also expected to permit phylogenetic linkage of key events in cereal evolution and provide new insights into domestication processes (Kresovich *et al.*, 2005).

Moreover, from the perspective of physiological and ecophysiological studies, sorghum is a relatively easy plant to grow and observe, particularly semi-dwarf sorghums that are 1-1.5 m tall, and have big leaves and panicles. This is particularly true for the study of tillering, the dynamics of which in sorghum is similar to that of other cereals (wheat, barley or rice) but easier to monitor because of larger organ size and lower tiller number, as modern varieties produce only a few primary tillers and virtually no secondary tillers.

Role of tillering in sorghum domestication and breeding strategies

Sorghum (*Sorghum bicolor* L. Moench) is the 5th most important cereal crop after rice, wheat, maize and barley (FAO statistics, 2006). It is the dominant crop in some farming systems of subtropical and semi-arid regions of West Africa, India, and North East Australia, that are prone to long periods of drought and where it provides food, fibre, fuel and feedstocks.

The ability to produce grain under adverse conditions, particularly in drought prone regions, makes sorghum an important "failsafe" source of food, feed, fibre and even fuel (Gnansounou *et al.*, 2005; Tuck *et al.*, 2006) in agro-ecosystems such as West Africa.

Sorghum is a more recently domesticated cereal than the other major grass crops and despite ongoing breeding programmes using diverse germplasm, comparatively little of the genetic diversity residing in the species and its wild relatives has been captured (Dillon *et al.*, 2007). Initial domestication of sorghum changed wild type seed characteristics to improved types with larger, non-shattering seeds. Disruptive selections resulted in sorghum types with vastly different characteristics in height, inflorescence type and end use (food, fodder, fibre, biofuel, etc). The *Sorghum* genus as currently defined consists of 25 subspecies (USDA-ARS, 2007) and is separated into five taxonomic subgenera: *Eu-Sorghum, Chaetosorghum, Heterosorghum, Para-Sorghum* and *Striposorghum* (Garber, 1950). *Eu-Sorghum* contains all cultivated sorghum races and varieties such as *Sorghum bicolor* subsp. *bicolor*, as well as the wild and weed species such as *S. halepense* (L.) Pers. or *S. arundinaceum* (Desv.) Stapf. (the known progenitor of *S. bicolor*) (Harlan and de Wet, 1971). All *S. bicolor* subsp. *bicolor* have 2n = 20 chromosomes, and are described as annual plants, with thick culms, often branched with many tillers. They were classified into five main races: *bicolor, guinea, caudatum, kafir* and *durra*. Analysis of genetic diversity in sorghum landraces and core collections based on latitude of origin, photoperiod sensitivity, seed and panicle characteristics, agronomic traits and DNA markers demonstrated that sorghum has considerable polymorphism that has been poorly exploited in terms of crop improvement (Deu *et al.*, 2006; Wu *et al.*, 2004).

During the second half of the 20th century, sorghum breeding programs progressed rapidly in countries with mechanised agriculture (USA, Australia, Brazil) (Smith and Frederikson, 2000; Jordan *et al.*, 2006). Hybrid cultivars were developed by using male sterility and restorer lines to avoid selfing, resulting in heterosis and significant improvement in phenotypic traits such as yield, plant height and days to flowering (Reddy *et al.*, 2006).

Yield advance was based on developing photoperiod-insensitive, dwarfed hybrids with some pest and disease resistance traits (such as midge) to improve yield for mechanised cropping systems. Accessing genetic diversity from sorghum landraces from Africa, India and China for traits such as drought tolerance and pest and disease resistance, was facilitated by development of a conversion program, which removed major photoperiod sensitivity and height genes (Rooney and Smith, 2000).

Regarding tillering, sorghum has a smaller range of variation than other cereals, but a single additional fertile tiller has a great impact on yield (Bruns and Horrocks, 1984; Lafarge and Hammer, 2002a). Tillering ability in sorghum has frequently been de-selected in breeding programs: modern varieties grown in USA and Australia are grown almost as uniculms (hybrid cropping systems). Tillering is prone to high phenotypic plasticity, making selection for general adaptation difficult. In addition, sorghum is generally bred for water limited environments, where water saving through limited canopy development is crucial. This explains arguments for breeding in intensive cropping systems in such environments to limit tillering (Hammer, 2006). On the other hand, one of the most successful varieties in recent years in Australia is a high tillering hybrid (MR Buster), but this likely relates to the wide range of environments experienced in the production zone and the significant yield advantage associated with tillering in good seasons given the risk-avoiding agronomic practices of wide rows and low population density. Also, in West Africa many traditional varieties of sorghum have high tillering ability (over ten productive tillers, Kouressy *et al.*, 2008), as tillering is advantageous for multiple purpose sorghum in Africa, intended to produce straw for roof thatching and animal feeding, stalks for fences (or fuel production in the case of sweet varieties) while maintaining grain production for human consumption (Gnansounou *et al.*, 2005; Tuck *et al.*, 2006).

Tillering – generalities

(1) Tiller emergence and growth during vegetative phase

During the vegetative development of Poaceae species, the shoot apical meristem produces successive elemental structures called phytomers, consisting of a node, an internode, a leaf and an axillary bud. The seedling emerges as a single shoot (main stem) but has the ability to generate axillary shoots called tillers, thus increasing the number of apical meristems. The architecture of the shoot system is determined by the activity of the primary shoot apical system already present in the embryo, together with the activity of axillary meristems formed after seed germination (Shimizu-Sato and Mori, 2001). Axillary shoots have a structure similar to that of the shoot from which they arise. In cereals and herbage grasses, this process is called tillering (Langer, 1963). The leaves of primary basal tillers can also subtend axillary buds, which in turn may form secondary tillers; this process can be repeated many times (tertiary tillers etc), resulting in a topological hierarchy of shoots.

Detailed histological observations of a tiller axis on rice show an apical meristem, meristematic phytomers with leaf primordia, one phytomer with a leaf elongating, one phytomer with an emerging leaf, and three or four phytomers with adult leaves and bearing nodal roots (Yang *et al.*, 1998). This structure is almost universal among species and tiller orders within a plant, with slight variations of the position of the youngest phytomer bearing roots (Klepper *et al.*, 1984). Following germination, tillers arise in succession from the base upwards, but genotype and environment determine which bud is the first to grow out. Two types of tillers can be distinguished: tillers growing upwards within the sheath of the subtending leaf called axillary or intravaginal tillers (which is the case of most cereals including sorghum) or horizontally bursting through the base of the leaf sheath called extravaginal tillers (as in *Agropyron repens* or *Poa trivialis*). Axillary tillers emerge from

inside subtending, green sheaths, maintaining the alternate positions of the successive leaves (Jewiss, 1972), whereas extravaginal tillers break through old sheaths and form a right angle with their mother tiller (Lopez *et al.*, 1967).

(2) Cessation of tillering and productive tillers

The number of tillers and the duration of tiller production differ greatly among cereal species. Tiller number per plant can range from none up to many in field conditions. The potential tillering ability of some grasses (barley, wheat, rice) even under unfavourable conditions remains superior to sparsely tillering species grown in optimal environments. Cessation of tiller emergence and stem elongation was reported to be connected in millet (Ong, 1984) and in wheat (Kirby *et al.*, 1985). Ballaré *et al.* (1987) also observed a reduced red:far-red ratio for LAI values close to 1 which was concomitant with a reduction in tiller production. In wheat, tillering ceased at specific light conditions within the wheat canopy, independent of population density and Evers *et al.* (2006) suggested that cessation of tillering is induced when the fraction of PAR intercepted by the canopy exceeds a specific threshold and red : far-red ratio drops below 0.35-0.40.

In sorghum, cessation of new tillers was reported to occur around a stable leaf area index (Lafarge *et al.*, 2002), whereas in millet, the rate of tiller abortion was related to LAI of the main shoot (van Oosterom *et al.*, 2001). A modern sorghum hybrid commonly produces zero to four fertile tillers, depending on the genotype and environmental conditions (Hammer *et al.*, 1993). Also fertile tillers can contribute to up to 60% of total plant leaf area and, depending on crop population density, contribute from 5% to 80% of grain yield (Lafarge *et al.*, 2002).

Genetic control of tillering

Tillering in grasses occurs in a two stage process: the formation of an axillary bud at each leaf axil and its subsequent outgrowth. The molecular mechanism of tillering remains to be elucidated. However, genes controlling shoot branching have been described in several species, including tomato, pea, maize and *Arabidopsis* (Ward and Leyser, 2004). They were classified into three groups (Shimizu-Sato and Mori, 2001) on the basis of whether they affect meristem initiation (e.g., genes *revoluta*, *pinhead*, *lateral suppressor* or *blind/torosa*), meristem outgrowth (e.g. *more axillary growth* [*max*], *ramosus* [*rms*] or *decreased apical dominance* [*dad*]) or both initiation and outgrowth (e.g. *supershoot/bushy* or *Teosinte branched1* [*Tb1*]). Several branch-regulating genes are conserved between monocots and dicots. Characterisation of the *Ls* gene of tomato in axillary meristem initiation revealed that it encodes a putative transcription factor of the GRAS family (Schumacher *et al.*, 1999). In *Arabidopsis*, *LAS* is an orthologous gene to tomato *Ls* gene that reduces the number of axillary shoots, whereas the expression of the *shoot meristemless* (*STM*) meristem marker gene correlates with the down-regulation of *LAS* expression (Greb *et al.*, 2003). Li *et al.* (2003) reported the isolation and characterization of *MONOCULM 1* (*MOC1*), a gene that is important in the formation of rice tiller buds. *MOC1* encodes a putative GRAS family protein that is expressed mainly in the axillary buds of rice and initiates axillary buds and promotes their growth. *MOC1* gene orthologous to *Ls* is expressed in a small number of epidermal cells in the leaf axils before any visible morphological meristem is present. These expression patterns of orthologous genes suggest that the basic control mechanism in the process of axillary bud development is conserved among tomato, *Arabidopsis* and rice (Ward and Leyser, 2004).

In monocots, contrasting branching phenotypes from maize and its wild progenitors from the teosinte group of the *Zea* genus allowed identification of the *Tb1* gene as a regulator

of branching (Hubbard *et al.*, 2002). *Tb1* is a member of the TCP (*Tb1 Cycloidea* PCF-domain protein) family of DNA-binding transcriptional regulators expressed in axillary meristems and in stamens of ear primordia. *Tb1* functions as a negative regulator of shoot branching. A *OsTb1* gene orthologue of *Tb1* was found in rice (Takeda *et al.*, 2003) but it is not yet clear whether *Tb*-related genes have a similar role downstream of *LAS/MOC1* in dicot branch development.

In sorghum, several QTLs that control variation in morphological traits, including tillering related traits (such as tiller number and the height of basal tillers) were identified in recombinant inbred populations or in inter- and intra-specific sorghum populations (Feltus *et al.*, 2006; Hart *et al.*, 2001; Lee, 1996). Other QTLs related to simple morphological traits such as plant height (Pereira and Lee, 1995) or complex traits such as stay-green (Borrell *et al.*, 2000; Xu *et al.*, 2000) or yield (Hart *et al.*, 2001; Quarrie *et al.*, 2006) were reported to be associated with tillering. However, the physiological basis on how those traits are interconnected, the functions of genes underlying the identified QTLs and their probable relation with genes discovered in other species are not understood.

Physiological control of tillering

Tiller production is related to meristematic activity that is influenced by numerous genotypic and environmental factors. Environmental factors affecting tiller initiation are most known to affect plant developmental events, such as radiation quantity and spectral quality, adequacy of nutrition, extent of oxidative stress, and presence of growth inhibitors. There are currently three main factors commonly accepted to explain tiller production:

- apical dominance
- photoperiod sensitivity mediated by the red:far-red(R:FR) ratio in the incident radiation

- nutrient (resource) availability

In the following sections, each of these factors is reviewed and discussed separately; a unified regulatory mechanism interconnecting those factors to explain tillering process is yet to be established.

Apical dominance

In many plant species, the activity of axillary meristems is inhibited by the primary shoot, a phenomenon known as apical dominance. This term was first used by Thimann and Skoog (1933) to describe the control exerted by the shoot apex over the outgrowth of lateral buds, whereby removal of the apical meristem stimulated growth of side shoots.

Apical dominance is mediated by a network of hormonal signals. Auxin (indoleacetic acid, IAA), produced in the apical meristem and in young expanding leaves, has a key role. The hypothesis of a direct action of auxin suggests that it inhibits lateral bud growth, but it may also affect bud outgrowth indirectly by mobilizing resources for already differentiated meristems (Phillips, 1975). A second group of hormones, cytokinins, was shown to promote cell division and lateral bud outgrowth (John *et al.*, 1993) by mobilizing plant nutrients (Fetene and Beck, 1993). The most widely accepted hypothesis on hormonally controlled apical dominance is based on the auxin/cytokinin ratio: auxin produced by the apical meristem and adjacent young leaves would block the utilization of root-synthesized cytokinin within lateral buds, thereby inhibiting their growth. Although the exact mechanism involved has not been resolved, Bangerth *et al.* (2000) have demonstrated auto-inhibition of auxin transport from lateral buds and showed that application of cytokinin to the dominated meristems could induce growth and change the order of dominance. They also showed that auxin exerts control on the production of cytokinin by roots and in turn, cytokinin may increase auxin production by stimulating lateral bud outgrowth, resulting in a feedback loop.

Tiller initiation in grasses is not consistently stimulated following defoliation or apical decapitation. Selective removal of the apical meristem while the leaves remain intact does not consistently stimulate tiller initiation, suggesting that auxin control over lateral bud outgrowth is rather indirectly inhibiting through control via some other factor (Dun *et al.*, 2006). Hence, although evidence for the hormonal mechanism of apical dominance is demonstrable and relevant, it seems to be too restrictive to explain all tillering processes.

Other types of signals can be involved in apical dominance and tillering inhibition. For example, it was reported that under phosphorus (P) deficit, tillering ability is reduced (Rodriguez *et al.*, 1998; Ming *et al.*, 2002), in particular because of signals coming from roots once P deficiency is sensed (Burleigh and Harrison, 1999; Luquet *et al.*, 2005a; Ming *et al.*, 2002; Rodriguez *et al.*, 1998; Shane *et al.*, 2003). Such signaling system involves soluble sugars not as a metabolic resource but as a chemical signal, because asimilates are not necessarily limiting during the onset of plant response to P deficiency (Shane et al., 2003; Liu et al. 2005; Luquet et al., 2005a).

Photosensitivity to the Red:Far-Red (R:FR) light ratio

Plants have mechanisms that enable them to respond to the presence of neighbouring plants. Photo-morphogenetic responses mediated by phytochrome responses to the R(600-700nm):FR(700-800nm) ratio provide plants with a shade-avoidance mechanism (Smith, 1982). The photosynthetic pigments, chlorophylls and carotenoids, absorb light over most of the visible spectrum. Radiation in the FR region is very poorly absorbed and consequently, the light that is reflected from vegetation is poor in R and enriched in FR wavelengths. Hence, the reflected R:FR ratio of photon irradiance is an important parameter describing the light environment in a canopy (Franklin and Whitelam, 2005).

The involvement of photo-morphogenetic processes in the control of tillering has been suggested by a number of authors (Casal *et al.*, 1985; Deregibus and Sanchez, 1983) and

the possibility of phytochrome participation was supported by the effect of R and FR treatments. Holmes and Smith (1975) showed that plant density affects the R:FR ratio, with phytochrome enabling the plant to detect shading and adapt its metabolism and development. Small changes in R:FR cause large shifts in the phytochrome photo-equilibrium (Pfr/Pr). Phytochrome is synthesized in the red-light absorbing form (Pr – maximum absorption around 660nm). Although this form is biologically inactive, upon photo-conversion to the far-red light absorbing form (Pfr – max around 730nm) it becomes active. The relative amounts of R and FR in incident radiation is translated by the phytochromes into different relative concentrations of the active Pfr form. The predominant effects of low R:FR are increased organ extension (elongation growth rate of stems and petioles) and the architectural modifications are accompanied by elevated leaf angles and an increase in apical dominance, leading to reduced branching in dicots and reduced tillering in grasses (Casal *et al.*, 1986). Yanovsky *et al.* (1995) also showed that FR exposed leaves reduced dry matter and structural carbohydrate accumulation, thus suggesting that phytochrome would have a direct effect on leaf growth and carbon partitioning.

However, the effect of the R:FR ratio on tillering rates in grasses is not straight forward. Certain species are responsive to this ratio while others are not (Deregibus *et al.*, 1985), and R:FR responsiveness within a species may be related to development stage. The R:FR, a signal to conveying the extent of canopy cover or leaf density, interacts with other signals related to resource availability (eg. water, carbon assimilates, nutrients) to determine the rate of tiller formation or death (Deregibus *et al.*, 1985). The result is a tiller population whose dynamics vary with resource availability and inter-plant competition. The R:FR ratio would thus have less impact on early tiller outgrowth (i.e. tiller outgrowing when crop cover is still low and competition for resources low, Lafarge *et al.*, 2002) than on late-tiller onset (potentially growing once canopy is already closed).

Some researches suggested that nutrition has a greater effect on tillering than the R:FR ratio. Monaco and Briske (2000) found that organ extension and tiller appearance were both most responsive to soil volume explored by roots while only organ extension responded to photosynthetic flux density (PFD) and responded weakly to R:FR ratio, suggesting that soil nutrient limitation strongly determines tillering.

Nutritional resource availability

The availability of minerals, water and carbohydrates shifts continuously in the plant as they are used by growing tissues or recycled from the dieback of live structures. Liebig (1863) first stated that plant growth is determined by the most limiting nutrient or resource. Although it is now generally accepted that plant growth can be limited by the availability of individual resources, studies on tillering have largely neglected these factors and rather focused on the hormonal control of tillering (cf. 'apical dominance' §).

The concept of resource based control of tillering consists of sink (i.e. growing tissues or organs) demand satisfaction depending on the supply available to the plant; it is commonly quantified by an evaluation of plant internal competition for resources among sinks such as outgrowing buds, equal to the ratio between supply and demand (Dingkuhn *et al.*, 2005; Dusserre *et al.*, 2002; Poorter and Nagel, 2000). Consequently, the apical dominance principle is inherently involved here as a sink to be satisfied in priority. Evidence that nutrient competition plays a major role in apical dominance was provided by Gregory and Veale (1957) who showed that in flax (*Linum usitatissimum* L.), the inhibition of axillary buds by apical dominance could be quite precisely controlled by varying nitrogen (N) and carbohydrate (C) supply. In experiments with *Pisum sativum* the negative effect of humidity on apical dominance interacted with the availability of both N and C (McIntyre, 2001): as long as the nutrient requirements of the existing shoot meristems exceed supply, lateral bud

inhibition is maintained. Once nutrient supply exceeds demand of existing meristems, demand for lateral bud outgrowth can be satisfied.

Main nutrients controlling tillering

Plant morphogenesis response to resources involves complex interactions of many substances. However, under most growing conditions, a relatively small number of resources plays a major role. The three major ones - water, nitrogen (N) and carbohydrate (C) - will be discussed here.

Water is crucial for all growth processes including cell expansion and metabolic activity ((Kramer and Boyer, 1995). With global climate change issues and important agricultural regions under diminishing water supply (Cassman, 1999), drought tolerance is essential to provide yield stability. Among secondary traits involved in plant response to drought, leaf expansion rate was shown to be particularly affected by drought (Casadebaig *et al.*, 2008; Lecoeur *et al.*, 1996; Tardieu *et al.*, 2000), in turn affecting plant leaf area development and also tiller outgrowth (Asch *et al.*, 2005; Dingkuhn *et al.*, 1990; Dingkuhn, 1996). Water deficit affects both assimilate sink and source processes, as well as nutrient uptake.

Nitrogen is the mineral nutrient that most frequently limits plant growth. It is contained in proteins, including Rubisco, the most abundant protein in plants in quantitative terms, and thus limits photosynthesis when in deficit. Tiller initiation in barley grown in a low-N medium is restricted but restored at any time by N addition (Aspinall, 1961). Decapitated ryegrass plants grown with high N availability produced more secondary tillers (Laidlaw and Berrie, 1974). The role of N in growth potential is emphasized by the relationship between leaf N content (Specific leaf nitrogen, SLN) and photosynthetic rate (Cruz, 1995). Nitrogen plays a key role in determining a plant's nutritional status, thereby at least indirectly regulating lateral bud outgrowth and other growth traits (Dingkuhn et al., 1990).

Carbohydrate resource (C) resulting from photosynthetic activity, driven by absorbed light, is not a limiting factor for shoot growth at high light intensities. However, the extent of assimilate accumulation in young plants is tightly dependent on extent of light interception, and hence leaf area production (Lafarge and Hammer, 2002b). Despite its abundance as a plant constituent, the non-photosynthetic or heterotrophic organs of a plant are permanently competing for assimilate throughout crop development (Dingkuhn *et al.*, 2006a; Dusserre *et al.*, 2002; Lechaudel *et al.*, 2005). The regulation of this competition represents a key process controlling plant morphogenesis, i.e. phenotypic plasticity (Dingkuhn *et al.*, 2005; (Dusserre *et al.*, 2002; Geigenberger *et al.*, 2005; Granier and Tardieu, 1999; Poorter and Nagel, 2000).

Resource driven plant phenotypic plasticity

The capacity of a plant to regulate morphogenesis and thereby optimizing the acquisition and use of a limiting resource was first addressed through the concept of 'plant functional equilibrium', showing for example that a plant grown in water limited conditions prioritizes root rather than shoot growth (Brouwer, 1962, 1983; Poorter and Nagel, 2000; Reich, 2001).

Although it has been established that resource availability affects tillering (Aspinall, 1961; Laidlaw and Berrie, 1974); Lafarge and Hammer, 2002a; Dingkuhn et al. 2006b), there is also evidence that nutrients do not act as direct cues for lateral bud activation. Measurements of tissue nutrient content before and after bud outgrowth did not demonstrate a clear correlation (Phillips, 1975). Cline (1994) showed that direct supply of nutrients to inactive lateral buds of *Arabidopsis* did not stimulate their outgrowth. This suggests that nutrient supply stimulates other growth promoters which in turn stimulate lateral bud outgrowth. Indeed, a number of experiments demonstrated that meristem activation or inhibition is regulated by nutrient based signals such as apoplastic, soluble carbohydrate concentration (Black *et al.*, 1995a; Liu *et al.*, 2004b; Rolland *et al.*, 2006a), other chemical

signals (Liu, 2004; Liu *et al.*, 2005a) or nitrogen (Kobayashi *et al.*, 2002), acting as secondary messengers. Also recently, the analysis of phenotypic plasticity (Dingkuhn, 1996; Dingkuhn *et al.*, 2006a; Luquet *et al.*, 2005a; Wright and McConnaughay, 2002) reintroduced and revisited the concept of functional equilibrium, defining phenotypic plasticity as the capacity of a plant to adjust its functional – structural relationship to its environment. This implies both short term (e.g. signaling at meristem level, (Black *et al.*, 1995b; Kobayazi *et al.*, 2002) and medium term responses (i.e. growth regulation, (Chenu *et al.*, 2007a; Luquet *et al.*, 2005a). The change in carbon partitioning between root and shoot in response to drought, N deficiency, P deficiency or reduced light intensity is a well-known example of this phenomenon (Bos and Neuteboom, 1998a, b; Dusserre *et al.*, 2002; Geigenberger *et al.*, 2005; Lechaudel *et al.*, 2005; Luquet *et al.*, 2006a; Reich, 2001; Yan *et al.*, 2004).

Tillering control by plant internal competition for carbohydrates

It has been stated in a number of earlier studies on wheat (Friend, 1965), rice (Honda and Okajima, 1970); (Dingkuhn *et al.*, 2006a; Dingkuhn *et al.*, 2006b; Luquet *et al.*, 2006a), barley (Kirby and Faris, 1972) and ryegrass (*Lolium perenne* L.) (Ong and Marshall, 1979) that tillering is regulated by plant internal competition for C. A tiller would thus emerge and develop under conditions of assimilate surplus once existing sinks (expanding organs) are satisfied, and cease its development or senesce under conditions of assimilate shortage (Dingkuhn *et al.*, 2005; Lafarge and Hammer, 2002a; Luquet *et al.*, 2006a). This *a priori* natural way of considering tillering response to C supply/demand balance, however, is biologically complex and relies on hypotheses that were or are still hard to validate experimentally:

(i) Plant C demand in a given period depends on aggregate sink activity exerted by all growing organs of the plant at a given time, a term that contains many unknowns and thus is difficult to estimate experimentally. Recently, several modelling approaches

aimed at computing C demand as the sum of plant organ growth in a given time window (Dingkuhn *et al.*, 2005; Dingkuhn *et al.*, 2006b; Luquet *et al.*, 2006a; Yan *et al.*, 2004). Demand was recently physiologically related to cell metabolic activity in expanding tissues, in particular to the activity of invertase enzymes (hydrolysing sucrose in glucose and fructose, key substrates for cell functioning) (Liu *et al.*, 2004a, 2005b; Roitsch *et al.*, 2000; Yang *et al.*, 2003).

(ii) Supposing that the plant is able to measure its overall sink-source ratio, and thus to estimate its ability to 'afford' new outgrowth, it may adjust C demand by reducing conditional sinks such as new tillers. This implies that the plant can sense when the level of competition for C resource becomes critical and when it has to regulate sink activity (i.e. morphogenesis); this hypothesis was recently demonstrated in several studies on the role of **sugar signalling processes** in regulating sink activity (Black *et al.*, 1995b; Bonfig *et al.*, 2007; Liu *et al.*, 2005b; Rognoni *et al.*, 2007; Rolland *et al.*, 2006b; Shane *et al.*, 2003; Stitt *et al.*, 2007). Meanwhile, recent study on rice suggested that the degree of sensitivity of tillering (considered among other morphogenetic processes) to available resources through sugar signalling, and thus to competition for C, could be **genotype dependent** (Dingkuhn *et al.*, 2006b; Luquet *et al.*, 2006a; Luquet *et al.*, 2007).

Role of modelling in the physiological and genetic study of complex traits

Application of crop models to molecular genetics and breeding

Models in biology are tools to bridge the gap between reductionism and holism (Hammer, 1998; Hammer *et al.*, 2005). Crop modelling developed since the early 1970s (de Wit, 1970) was initially viewed as a means to describe and predict phenomena at one level of biological organization (plant or crop) by integrating responses at the lower explanatory level (organ or

plant). Quantitative descriptions of the relationships between scales were the basis to explore theories and develop explanations of how plants and crops behave, taking into account the interaction between components such as growth and development. This approach has been continuously supported by advances in crop physiology and computing technology. However, until recently, most crop models reflected current research and application priorities while drawing comparatively little from new knowledge in crop physiology and genetics, e.g. SARRA-H, (Dingkuhn *et al.*, 2003); APSIM (Agricultural Production Systems sIMulator) (Wang *et al.*, 2002); STICS (Simulateur mulTIdisciplinaire pour les Cultures Standard), (Brisson *et al.*, 2003).

In the last decades, the genomic revolution generated a huge amounts of genetic information opening up new research areas for crop improvement and breeding. Indeed, traditional breeding approaches for improving abiotic stress tolerance have had limited success (Richards, 1996). This can be partly explained by the fact that traits such as yield or leaf area index and biomass are integrative and related to a large number of genes, and are thus strongly influenced by genotype x environment x management interactions. By focusing on component traits that contribute to complex trait expression, however, it would be possible to deal successively with a smaller number of genes and thus take more advantage of genetic information now available (Hammer et al., 2002; Dingkuhn *et al.*, 2005). Moreover, it would facilitate the introduction of one desired trait from one closely related species to another (Tester and Bacic, 2005).

In this context, crop physiology has regained significant interest in recent years as a way to dissect complex traits into elemental processes easier to study and to relate to genetic information (Hammer *et al.*, 2002, 2006; (Dingkuhn *et al.*, 2005; Reymond *et al.*, 2004; Tardieu *et al.*, 2005). As a consequence, the opportunities offered by modelling were also revisited:

(i) Modelling is useful to **formalize component trait control by G and E**, based on equations with genotype dependent parameter values that can be considered as 'genotypic process based traits', presumably less prone to GxE than the resulting, computed or observed phenotypic trait (Chapman *et al.*, 2000; Chapman *et al.*, 2002; Dingkuhn *et al.*, 2005; Tardieu *et al.*, 2005; Whish *et al.*, 2005). **Model parameters thus became particularly relevant as they gradually became more directly related to QTL or genes** (QTL detection, association studies), instead of, or complementarily to, directly observed complex traits. This approach, called **model assisted phenotyping,** relies on the use of reasonably simple models or even only on modules or equations formalizing one biological process (i.e. dealing with a small number of parameters). This may require parameter optimization tools (algorithms) to apply models in an inversed mode (heuristic approach) and fit parameters to phenotypic data observed on individual genotypes in a population or a core collection (Dingkuhn *et al.*, 2005; Hammer *et al.*, 2002a; Luquet *et al.*, 2006a). Model assisted phenotyping was conceived in some way to play a role similar to purely physiological approaches (e.g. 'omics' sciences, (Gibon *et al.*, 2004) that also aim at dissecting complex traits in elemental processes, but with the constraint to be time consuming and expensive.

(ii) Modelling is also seen as the only way to **integrate component traits and compute their combined impact on complex trait expression,** e.g. biomass, leaf area and yield (Chapman *et al.*, 2002; Cooper *et al.*, 2002a; Cooper *et al.*, 2002b; Hammer *et al.*, 2002a; Whish *et al.*, 2005; White and Hoogenboom, 2003; White, 2006; Yin *et al.*, 2004). Such model application enables to explore *in silico* the adequacy of traits or even allele combinations (if already linked to model parameters) for a given environment, test ideotypes and guide further research, e.g. on breeding strategies ((Chapman *et al.*, 2003); Dingkuhn *et al.*, 2005; (Chenu *et al.*, 2007a, b; Hammer *et al.*, 2002b).

(iii) **Crop models were recently used to analyze target populations of environments (TPE)** a breeding program has to deal with (Chapman *et al.*, 2003; Heinemann *et al.*, 2007; Kouressy *et al.*, 2008): for this purpose, a crop model is run across the environmental conditions observed within a breeding region (sensitivity analysis using multi-site and multi-annual data, and parameter settings for one or several reference genotypes). The objective is to develop a typology of environments impacting on the crop, and on that basis to optimize breeding and selection strategies.

(iv) **Modelling opportunities described in (i, ii, iii)** have been integrated into an operational sorghum breeding program in Australia (Hammer and Jordan, 2007) and were recently explored in a project (Whole Plant Modelling project, 'WPM', May 2005 – May 2008, proceedings available at http://www.generationcp.org/research.php?da=0898003) within the Generation Challenge Program (GCP, international consortium dealing with crop improvement for drought tolerance through genomics research). Other projects involving modelling based on similar concepts are under way at CIRAD, APSRU and many other research groups.

Modelling sorghum tillering as driven by plant internal carbohydrate availability

Efforts were made during the last decades to increase the functionality of crop/plant models, such that GxE interaction for tillering would be an emergent consequence of the regulation through morphogenesis (cf. PMA 2003 and 2006 and FSPM 2004 and 2007 congresses), but only a few attempts were made to model tillering regulation by C supply/demand status. Lafarge and Hammer (2002a, b) proposed a model to predict tillering dynamics in sorghum, with emphasis on tiller emergence dynamics, cessation of tiller emergence and decrease in number of potentially fertile tillers. They identified a hierarchy in tiller fertility similar to that of Canell (1969) for barley and outlined concepts related to leaf

area development and internal plant competition for C as a means to quantify tiller dynamics and abortion at whole plant scale. Dingkuhn *et al.* (2001) observed in rice that tiller production and abortion strongly depends on carbon resources. They more recently proposed a model formalizing in a simple way the response of meristem activity to plant internal competition for C resources (supply : demand ratio) to simulate plant vegetative morphogenesis and its plasticity (Luquet *et al.*, 2006b).

PhD OBJECTIVE

Problem statement

In the general introduction, tillering was defined as a key trait influencing yield of cereals and of sorghum in particular, and as a complex trait depending on both environmental and genetic determinisms. Further, sorghum was identified as a particularly relevant species for genetic and ecophysiological studies of tillering.

Among the factors controlling tillering, plant internal competition for carbohydrate resources was shown to be crucial, although difficult to study because of its complex role in plant phenotypic plasticity as both substrate and chemical signal (Lafarge and Hammer, 2002; (Dingkuhn *et al.*, 2005; Dingkuhn *et al.*, 2006b; Luquet *et al.*, 2006a); (Liu *et al.*, 2005b; Rognoni *et al.*, 2007; Rolland *et al.*, 2006b; Shane *et al.*, 2003). Sugar availability, as an expression of sink-source relationships, is therefore thought to act as a link with resource driven morphogenetic processes, such as tillering.

It is possible to simulate tillering and other complex, resource dependent, morphogenetic processes with physiological plant models. This has for example been attempted with *EcoMeristem*, a model developed to simulate the environmental and genotypic components of the control of morphogenetic processes. In theory, such models can also be used heuristically to analyze the genetics of these processes.

Using complex numerical models for heuristic applications, however, has its own risks because requirements in terms of model accuracy and parameterization methodology are very high. This experimental study therefore approaches the phenomenon of tillering and its environmental (E) and genetic (G) control through a conceptual model, based on the general hypothesis that tillering of sorghum is a genotype dependent function of source/sink (supply/demand) relationships within the plant.

Objective

The objective of the present work is to develop a conceptual modelling framework formalizing the E and G control of tillering in sorghum, based on the general hypothesis that assimilate resources of the plant have a strong influence on tillering. The hypothesis will be tested both with respect to E effects on tillering (several controlled and field environments to generate contrasting C supply/demand situations) and G effects (comparison of 6 contrasting genotypes). Once validated, the conceptual model will be applied to (1) support a genetic study on sorghum mapping populations, aiming at identifying quantitative trait loci (QTL) controlling tillering ability; and (2) improve and test existing crop models capable of simulating tillering, such as *EcoMeristem* (Luquet *et al.*, 2006) and APSIM (Wang *et al.*, 2002).

Organization

The thesis is organized in six chapters: The introduction (I), followed by four chapters presenting and discussing results in the form of scientific articles (II, III, VI, V), and a general discussion and conclusion (VI).

Among the chapters in the form of scientific articles, **Chapter II** (submitted to Annals of Botany) addresses the environmental control and **Chapter III** (submitted to Annals of Botany) addresses the genetic control of tillering, based on a set of field and controlled

environment experiments aiming at elaborating the conceptual model for tillering control. **Chapter IV** (oral communication in 5th ICSC 2008; scientific article awaiting submission) presents an application of the conceptual model in the context of a genetic study on tillering (QTL analysis) for a set of 8 mapping populations. In **Chapter V** (precursor of scientific article to be submitted after thesis completion), modelling concepts are further investigated experimentally using observations on sugar content and partitioning in three contrasting genotypes during tillering. The validated conceptual model is used to improve, calibrate and test *EcoMeristem*. Similar work using the sorghum model of the APSIM platform is in progress, but less advanced, and will not be presented here.

Finally, **Chapter VI** presents a brief overall discussion of the results and the perspectives emanating, before finishing with the main conclusions.

"A farmer went out to sow his seed,
As he was scattering the seed,

Some fell along the path,
and the birds came and ate it up.

Some fell on rocky places,
where it did not have much soil.
It sprang up quickly, because the soil shallow.
But when the sun came up,
the plants were scorched,
and they withered because they had not root.

Other seed fell among thorns,
which grew up and choked the plants.

Still other seed fell on good soil,
where it produced a crop
a hundred, sixty or thirty times what was sown."

CHAPTER II

Regulation of tillering in sorghum: Environmental effects

Hae Koo Kim [1,2]

Graeme Hammer [1]

Erik van Oosterom [1]

Michael Dingkuhn [2]

Delphine Luquet [2]

Submitted to Annals of Botany

[1] The University of Queensland, School of Land, Crop and Food Sciences, Brisbane QLD 4072, Australia

[2] CIRAD, UPR 59, Avenue Agropolis Lavalette TA A 59/01 34398 Montpellier, France

CHAPTER II

ABSTRACT

• *Background and Aims* Tillering has a significant effect on canopy development and, hence, resource capture, crop growth, and yield in sorghum. However, the ecophysiological bases of tillering and its regulation by environmental and genetic effects are not fully understood. The objective of the first part of this study was to understand and quantify the environmental effects on tillering in sorghum.

• *Methods* A series of five experiments with a wide range in radiation and temperature conditions was conducted and detail of tillering responses for one representative hybrid was monitored. The concept of internal plant competition for carbohydrate was developed for this purpose.

• *Key Results* Tiller appearance was highly synchronised with main stem leaf appearance, with a consistent hierarchy for tillering across environments. The main environmental effect was on the frequency of tiller appearance, in particular of the lower-rank tillers. This explained some of the observed environmental differences on the onset of tillering. A generalised index of internal plant competition, that took account of plant assimilate supply and demand (S/D index), explained most of the variation in maximum tiller number observed across the five experiments.

• *Conclusions* This result was consistent with the hypothesis that internal plant competition regulates tillering in sorghum. Hence, the framework outlined has a predictive value that could provide the basis for dynamic simulation of tillering in crop growth models.

Key words: *Sorghum bicolor* L. Moench, tiller hierarchy, internal plant competition, carbohydrate supply-demand ratio, leaf area development, radiation, temperature, modelling

INTRODUCTION

Tillering is an important agronomic trait in many high-tillering cereals (e.g. wheat, rice, barley) and contributed significantly to improved yield associated with the 'green revolution' (Conway and Toenniessen, 1999; Khush, 1999). While tillering is considerably less in sorghum, it nonetheless has a major influence on plant leaf area development (Hammer *et al.*, 1993; Lafarge *et al.*, 2002) and, hence, crop water use patterns and adaptation to water-limited environments (Hammer *et al.*, 2006). Modern sorghum hybrids produce from zero to four fertile tillers in field conditions so that in some cases more than 60% of total plant leaf area and up to 80% of total grain yield can be attributed to tillers (Hammer *et al.*, 1993; Lafarge *et al.*, 2002).

The ecophysiological bases of tillering and its regulation by environmental and genetic effects are not fully understood. Hence, its formalisation in most crop models is limited. Indeed traditional crop models struggle to predict complex traits as there is a lack of understanding of the underlying component processes and genotype x environment interactions (Loomis *et al.*, 1979), such as those responsible for phenotypic plasticity of plant architecture, morphology and phenology (Wright and McConnaughay, 2002). In sorghum, early attempts at modelling were based on concepts considering tillers as similar to the main culm (Rosenthal *et al.*, 1989; Heiniger *et al.*, 1997). Later attempts allowed for differences in size among main culm and tillers (Hammer *et al.*, 1993; Hammer and Muchow, 1994) but remained descriptive and still required input of tiller number. Physiological dissection of such a complex trait can help in formulating models that are better suited to predicting consequences of environmental and genetic variation (Hammer *et al.*, 2002).

Early studies on leaf and tiller number dynamics in rice, wheat and barley, used site filling concepts (Katayama, 1951). Tillering was assumed to follow a fixed pattern with a definite regularity and interval in relation to main stem development (Bos and Neuteboom, 1998), but

specific site usage (i.e. fraction of buds that ultimately developed into a productive tiller at a specific site) remained subject to a number of hypotheses. Tillering is often regarded as an architectural trait (Doust, 2007) and considered important for its contribution to yield in high tillering species (Evans, 1993). It has been reported to be driven first by tiller site formation at the axil of every leaf and then by the number of these buds that outgrow into tillers (Li *et al.*, 2003). Studies on wheat (Friend, 1965), rice (Honda and Okajima, 1970), barley (Kirby and Faris, 1972), and ryegrass (Ong and Marshall, 1979) suggested that tiller outgrowth was dependent on resource availability for the crop (e.g. nitrogen and carbohydrate) but did not pursue this to quantification at individual plant level. Lafarge and Hammer (2002b) detailed how plant internal competition for carbohydrate could be applied to modelling dynamics of tiller fertility in sorghum and Dingkuhn *et al.* (2006) and Luquet *et al.* (2006) applied this concept to model rice morphogenesis based on the ratio between carbohydrate supply and demand (S/D). In these studies, S/D was shown to be a key factor controlling tiller emergence, growth and survival. Tillering is also known to be controlled by light quality via the red/far red (R/FR) ratio (Deregibus *et al.*, 1985; Casal *et al.*, 1986; Evers *et al.*, 2006) but studies in sorghum have suggested that this likely relates to timing of cessation of tiller outgrowth due to plant-to-plant competition rather than to initiation of tiller outgrowth associated with S/D (Lafarge and Hammer, 2002b).

The S/D ratio is a complex indicator of plant status that depends on both environmental and genotypic factors. Solar radiation and temperature are the main environmental factors that affect S/D. Radiation level is a key regulator of photosynthesis (Johnson *et al.*, 1995) and hence plant carbohydrate supply (Choudhury, 2001; Murchie *et al.*, 2005). By contrast, temperature has a major influence on developmental processes such as node and leaf production and expansion rates, which affect crop leaf area index (LAI) (Hammer *et al.*, 1993; Tardieu *et al.*, 1999; Mazzella *et al.*, 2000). The ratio of these two key environmental factors was defined by Nix (1976) as the photo-thermal quotient (PTQ), which is commonly used as

an environmental indicator of carbon supply for plant growth. A high PTQ indicates that more carbohydrate can be produced per developmental unit and suggests an excess of supply over demand. This concept has been used in quantifying environmental effects on grain number in cereals (Fischer and Wilson, 1975; Ortiz-Monasterio *et al.*, 1994). However, it does not consider the level of intercepted radiation by plants during growth.

The supply of carbohydrate during early vegetative development is also strongly dependent on plant leaf area dynamics as shown for sorghum by Lafarge and Hammer (2002). In the first few weeks after emergence until the panicle initiation stage, main stem leaf area is both the unique supplier and the main sink for carbohydrates, and total plant leaf area dynamics can be directly linked to tiller production (Hammer *et al.*, 1987). When examining allocation of carbohydrate among organs in plants, the timing of organ initiation and development needs to be considered, as it controls the simultaneous growth of competing sinks, as well as the relationship between photosynthetic sources and growing sinks (Wardlaw, 1968; Heuvelink, 1996).

In sorghum, environmental and genotypic controls of tillering have not been addressed comprehensively using the S/D framework. Lafarge *et al.* (2002) described a hierarchy for tiller emergence and fertility across a range of densities in a single environment for a specific genotype, but detailed physiological study on tillering across a range of environments and genotypes has not been reported.

The objective of this study was to understand and quantify the environmental effects on tillering in sorghum by considering the concept of regulation of tillering via plant S/D. A series of five experiments intended to generate a range in levels of plant internal competition for carbohydrate was used to explore responses of one representative hybrid. Genetic effects will be addressed in a companion paper (Kim *et al.*, unpublished).

TABLE 1: Summary of the environmental conditions in field (Exp1-3) and controlled environment (Exp4-5) experiments. PTQ indicates photo-thermal ratio for the specified period: PT=pre-tillering period; TEM=Tiller emergence period; TSEN=Tiller survival or senescence period; (#), (##) thermal time up to main stem L8 or L9 fully expanded stage in Exp4 and Exp5 (respectively)

	Exp1	Exp2	Exp3	Exp4	Exp5
Experiment site	Warwick HRS	Warwick HRS	Gatton UQ	Glasshouse (Montpellier)	Phytotron
Location (lat., long., alt.)	28°12'S, 152°5'E 462 m	28°12'S, 152°5'E 462 m	27°34'S, 152°18'E 94 m	43°38'N, 3°52'E 46 m	
Sowing date	25/10/2004	02/03/2005	16/01/2006	31/08/2006	Transplantation
Photoperiod at sowing	13h02	12h37	13h31	13h13	13h
Max daily radiation ($MJ.m^{-2}.day^{-1}$)	22.9	21.0	20.3	15.0	10.0
Average daily min temperature (°C)	13.1	11.9	19.9	21.2	22.0
Average daily max temperature (°C)	27.9	30.0	33.4	38.5	28.0
Average daily temperature (°C)	20.5	21.0	26.7	29.9	25.0
Average daily humidity	58.0	60.2	65.6	63.2	70.0
PTQ ($MJ.m^{-2}.day^{-1}$) [PT]	2.4	2.1	1.5	0.9	0.5
PTQ ($MJ.m^{-2}.day^{-1}$) [TEM]	3.3	2.6	1.3	0.6	0.7
PTQ ($MJ.m^{-2}.day^{-1}$) [TSEN]	1.9	2.3	1.4		
Cumulative radiation at flag leaf ($MJ.m^{-2}.day^{-1}$)	1401	1241	899	195#	245##
Cumulative thermal time at flag (°C d^{-1})	604	545	656	310#	407##

MATERIALS AND METHODS

Experimental details

A well adapted Australian sorghum hybrid (MR Buster: Midge Resistant Buster), known to be high tillering, was used in five experiments (three field experiments and two controlled environment experiments) that generated a wide range of plant S/D status (Table 1).

The field experiments were conducted over two summer growing seasons and differed in sowing dates to ensure a range in S/D levels (Table 1). Experiment 1(Exp1) was sown on 25 Oct. 2004 at Warwick (28°12'S, 152°5'E, elevation 462 m asl) in the sorghum belt of Eastern Australia. Because of the early sowing date, temperature was relatively low, but

radiation levels were average. Experiment 2 (Exp2) was sown at Warwick on 2 Mar. 2005 and experienced high temperature and radiation during the tillering phase. Experiment 3 (Exp3) was sown at Gatton (27°34'S, 152°18'E, 94 m asl) on 16 Jan. 2006 and encountered medium to high temperature and high radiation. Both Exp1 and Exp2 were conducted until anthesis, whereas Exp3 was carried through until physiological maturity.

Field experiments were laid out as a randomised complete block design with three replicates and up to seven entries. Only data for MR Buster are considered here; details associated with other genotypes are reported in the companion paper (Kim *et al.*, unpublished). Plot size was 4 rows of 15 meters, with a row spacing of 1 meter. The centre 2 rows were used for data collection. Experiments were thinned to a uniform stand of 50,000 plants per hectare before any mutual shading could occur, i.e. at about full expansion of the 3-4th leaf on the main stem. Each experiment received a basal fertiliser application prior to sowing and fertiliser and supplemental irrigation were managed to ensure optimum growing conditions throughout the experiments. Atrazine was applied after sowing, prior to emergence, to control weeds. Insecticides and fungicides were applied as necessary to control heliothis and rust. Air temperature (T, minimum, maximum and average with Campbell Scientific 108-L6), relative humidity and global solar radiation (Sradn in $MJ.m^{-2}.day^{-1}$ with Li-Cor Li200S) were measured at 1.5m above the soil surface. Data were recorded hourly using a datalogger (CR10, Campbell Scientific). Radiation interception was measured with two 1 m long tube solarimeters (Type TSL, Delta-T Devices) at ground level in each plot (one tube between rows and one across rows), plus a reference one outside the crop. Solarimeters were cleaned weekly and dead leaves removed to ensure that the radiation intercepted was due to green leaf only. The field experiments provided contrasting combinations of climatic variables (Table 1).

The two controlled environment experiments were carried out in a randomised complete block design with four replicates and six genotypes (including MR Buster) in a greenhouse (Exp4) and a phytotron (Exp5) at CIRAD research station in Montpellier, France

(43°38'N, 3°52'E, 46 m asl). The experiments focussed on early plant development and were terminated when 8-9 main stem leaves were fully expanded, around the end of the tiller emergence period. In Exp4, solar radiation was augmented with artificial halogen lamps during cloudy days to maintain at least 300 $\mu mol.m^{-2}.s^{-1}$ of photosynthetic active radiation (PAR). Radiation was measured with PAR sensors (PAR-IR/M SOLEMS, measuring energy of photons in the wavelength bands between 300nm and 700nm) in $\mu mol.s^{-1}.m^{-2}$ and then converted into global solar radiation units of $MJ.m^{-2}.day^{-1}$ (for comparison with field conditions). Temperature was controlled to maintain approximately 20ºC during the night and a cooling system was used when temperature exceeded 35ºC during the day. In Exp5, radiation was provided by artificial halogen lamps at constant intensity. However, mean daily irradiance varied with height (closeness to lamps) between 250 $\mu mol.m^{-2}.s^{-1}$ (during early growth) and 700 $\mu mol.m^{-2}.s^{-1}$ (at last harvest) with a fixed daily photoperiod (13 hours). Day and night temperature was set at 28ºC and 22ºC respectively and relative air humidity was between 60% and 80% (day/night). In both experiments, seeds were germinated for one day at 30ºC in an illuminated culture chamber, and subsequently transplanted in drained 1L pots containing fertilised soil. Pots were watered at least once daily to field capacity with a culture solution (pH 5.5) containing the following nutrients (concentrations in mM): KH_2PO_4=0.21, K_2HPO_4=0.06, KNO_3=1.98, $Ca(NO_3)_2$=2.96, $MgSO_4$=0.61, $(NH_4)_2SO_4$=0.53, $MnSO_4$=2.9×10^{-3}, $ZnSO_4$=2.5×10^{-3}, KCl=0.1, $(NH_4)_2MoO_4$=6×10^{-5}, $CuSO_4$=6.3×10^{-2}, H_3BO_3=7.4×10^{-3}, EDTA-Fe=0.206.

Plant measurements

Development

In field experiments, emergence was scored daily on 2m per row in each until complete emergence. Anthesis was scored on five adjacent tagged plants in the inner rows of each plot.

Each panicle was rated at least once a week until the beginning of anthesis, and then every two days by estimating how far down the panicle anthers were visible. The date of anthesis was determined in each plot when over 50% had exerted anthers midway down the panicle. Physiological maturity (Exp3 only) was scored on main stem panicles of ten plants per plot by screening for the presence of a black layer on individual grains. Physiological maturity was reached when 90% of the panicles had grains that had reached the black layer stage in the lower part of the panicle (Eastin *et al.*, 1973).

The number of visible, fully expanded, and senesced leaves on the main stem and each tiller were recorded weekly on the five tagged plants per plot in the field and on all plants of each replication in the controlled environment experiments. A leaf was visible once its tip was visible above the enclosing leaf whorl, fully expanded once its ligule was visible above the enclosing sheath of the previous leaf, and senesced if 50% or less of its surface was green. The average of the five plants in each plot gave the number of visible, fully expanded, and senesced leaves on each axis at a given date. Thermal time was calculated from a broken linear function of the hourly mean air temperature, using 11°C, 30°C, and 42°C as the base, optimum, and maximum temperatures (Hammer *et al.*, 1993). The average thermal time separating successive leaf tip (tip-phyllochron) or ligule (lig-phyllochron) appearance was determined as the slope of the relationship between leaf number and cumulative thermal time. Leaf Expansion Duration (LED) was subsequently computed for each leaf rank of the main stem (L_{rank}) as the time separating tip and ligule appearance.

Growth

In both field and controlled environment experiments, the fully expanded area of each leaf of each culm was estimated by non-destructive measurements of leaf blade length and maximal width on the five tagged plants in each plot in Exp1, 2 and 3 and on one plant per replication

in Exp4 and 5. Blade length was measured from the ligule to the tip and blade width was measured at its maximal point. Blade area was then calculated as the product of length and width and a shape coefficient, which was determined by linear regression of the blade area measured with a leaf area meter (either Delta-T or LI-COR LI-3100C for the field experiments, CID CI-203 for the controlled environment experiments) on the product of leaf length and width of the same leaf. The value of the shape coefficient (0.69) was similar to that reported by McCree *et al.* (1984) and Lafarge *et al.* (2002) for sorghum. However, to calculate plant leaf area during early growth accurately, values of 0.85 and 0.75 were used for main stem L1 and L2 respectively.

Aboveground organ biomass was determined by destructively sampling an area of 1 m^2 (ten plants) in each plot approximately once a week from thinning to anthesis (Exp1 and Exp2) and up to physiological maturity (Exp3). Plants were cut at ground level from the two inner rows of each plot. In Exp4 and Exp5, similar measurements were done on one plant per block at three stages: (1) before any tillers had emerged (before main shoot L4 fully expanded), (2) after first tiller outgrowth (around main stem L5 or L6 fully expanded), and (3) during tiller appearance (at L8 or L9 fully expanded). Each sample was separated into main stems and tillers, identified by their rank (see below). For each culm rank biomass was separated into four components: green leaves, dead leaves, stems (including sheaths) and panicles. Green leaf area was measured with a leaf area meter. Net above-ground dry weight for each component was obtained after drying samples at 80°C for at least 7 days. Plant or tiller specific leaf area (SLA, in $cm^2.g^{-1}$) was calculated by dividing green leaf area by its corresponding leaf dry weight. Leaf area index (LAI) was calculated for each axis as its total green leaf area divided by the harvest area of each plot.

Tillering characterisation

Tiller emergence was observed weekly on the five tagged plants in each plot and on the weekly harvested plants in field experiments and on all individual plants in the controlled environment experiments (Exp4 and 5). The rank of each emerged tiller was defined by the main stem node from which it developed and the sheath from which it emerged, with tiller 1 (T1) emerging from the sheath of main stem leaf 1. In our experiments, only primary tillers emerging from mainstem axillary buds were produced. Tillering was expressed relative to the number of visible or fully expanded main stem leaves. This represented a plant based index of developmental stage, similar to Haun stage (Haun, 1973) that was not biased by phyllochron variations across environments.

Maximum tiller number (TN_{max}) and fertile tiller number (FTN) were observed on plants used for continuous and destructive measurements. Using continuous observations on tagged plants, the distinction between fertile and arrested/senesced tillers was established for each tiller rank depending on whether at least one new leaf appeared between successive weekly observations (Lafarge *et al.*, 2002). TNmax was determined as the total of all emerged tillers, irrespective of whether they became fertile or not. FTN estimated in Exp1, 2 and 3 took into account tillers that continued to develop until anthesis and produce a panicle. Tiller rank fertility rate (expressed as a percentage) was calculated as the ratio of fertile tiller number to total tiller number for each tiller rank.

Development of a plant S/D framework

Data were analysed to identify a quantitative relationship between tillering and S/D indicators. First, we calculated PTQ as a general indicator of S/D during the key plant development phases: (1) pre-tillering [PT], (2) tiller emergence [TEM] and tiller senescence

[TSEN]. The PTQ is generally considered a broad-based environmental indicator of available solar radiation per unit of thermal time during a particular period

$$PTQ = \frac{\sum_{d_1}^{d_2} \frac{I_0}{TT}}{d_2 - d_1} \quad \text{in MJ.m}^{-2}.°\text{Cd}^{-1} \qquad (1)$$

With I_0: incident daily global radiation in MJ.m^{-2}.day^{-1}; TT: daily cumulated thermal time as the sum of hourly thermal time; d_2-d_1: considered period interval.

Secondly, a form of Relative Growth Rate (RGR – Eq. 2), which expresses plant dry weight gain per unit of existing dry weight, but per unit thermal time rather than calendar time, was compared with Relative Tillering Rate (RTR – Eq. 3) to check whether a robust linear relationship between those two variables could be found as noted for rice (Dingkuhn *et al.*, 2001).

$$RGR = \frac{[\overline{\ln(DW_2)} - \overline{\ln(DW_1)}]}{(TT_2 - TT_1)} \qquad (2)$$

RGR in g.g^{-1}.°Cd^{-1}, with DW_n plant dry weight at day n and TT_n the accumulated thermal time at day n.

$$RTR = \frac{[\overline{\ln(TN_2 + 1)} - \overline{\ln(TN_1 + 1)}]}{(TT_2 - TT_1)} \qquad (3)$$

RTR in tiller.tiller^{-1}.°Cd^{-1}, with TN_n plant tiller number at TT_n.

In classical growth analysis, RGR is calculated using DW_1 and DW_2 at times t_1 and t_2. The approach adopted here for RGR and RTR follows the method of Hoffmann and Poorter (2002) that uses means of natural logarithm transformed plant weights to avoid bias.

Subsequently, more specific indicators of carbohydrate supply (S) and demand (D) were calculated. Under non-limiting conditions, supply of assimilates to the crop is determined by the amount of radiation intercepted by the crop. At the whole canopy level, intercepted radiation is commonly calculated by accumulating the product of the hourly incident radiation and the fraction intercepted by the crop. The fraction of intercepted light was calculated by comparing light intercepted by solarimeters placed at ground level in the

crop with interception by reference solarimeters above the crop. However, in the very early stages of growth, we assumed that all leaf area was intercepting light (Lafarge and Hammer, 2002a), making supply proportional to leaf area (plant size), which can be indexed through the size of the last fully expanded leaf. To account for environmental differences in the duration of leaf expansion, the supply of assimilates needs to be adjusted for the duration of the developmental unit, which can be indexed through the LED. Environmental differences in LED for a particular leaf rank have been observed (for example, LED of L5 varied from 35°Cd to 54°Cd). The demand for assimilates by the crop can be related to the temperature-driven potential rate of leaf area growth (ΔLA), as stem elongation has not commenced at this stage. Therefore, a crop assimilate S/D_{index} that extends the PTQ concept and that could be tested for association with tillering can be represented by the generic formula:

$$S/D_{index} = \frac{I_o \times Leafsize \times LED}{\Delta LA} \quad (4)$$

RESULTS

Environment characterisation

The different sowing dates and locations (field and controlled environment) resulted in considerable variation in environmental conditions in terms of radiation and temperature. The PTQ experienced during early vegetative growth (PT-TEM) was similar in Exp1 and Exp2 with an average ratio of 2.5 $MJ.m^{-2}.°Cd^{-1}$ and 2.3 $MJ.m^{-2}.°Cd^{-1}$ respectively (Fig. 1). In Exp3, PTQ was lower (1.5 $MJ.m^{-2}.°Cd^{-1}$) because of the higher temperatures during early developmental stages. The low PTQ in Exp4 (0.6-0.9 $MJ.m^{-2}.°Cd^{-1}$) was due to low radiation and very high temperatures, whereas in Exp5 the very low radiation, combined with moderate temperatures, resulted in a PTQ that increased from 0.4 to 0.8 $MJ.m^{-2}.°Cd^{-1}$ as plants grew closer to the lamps with increasing plant size.

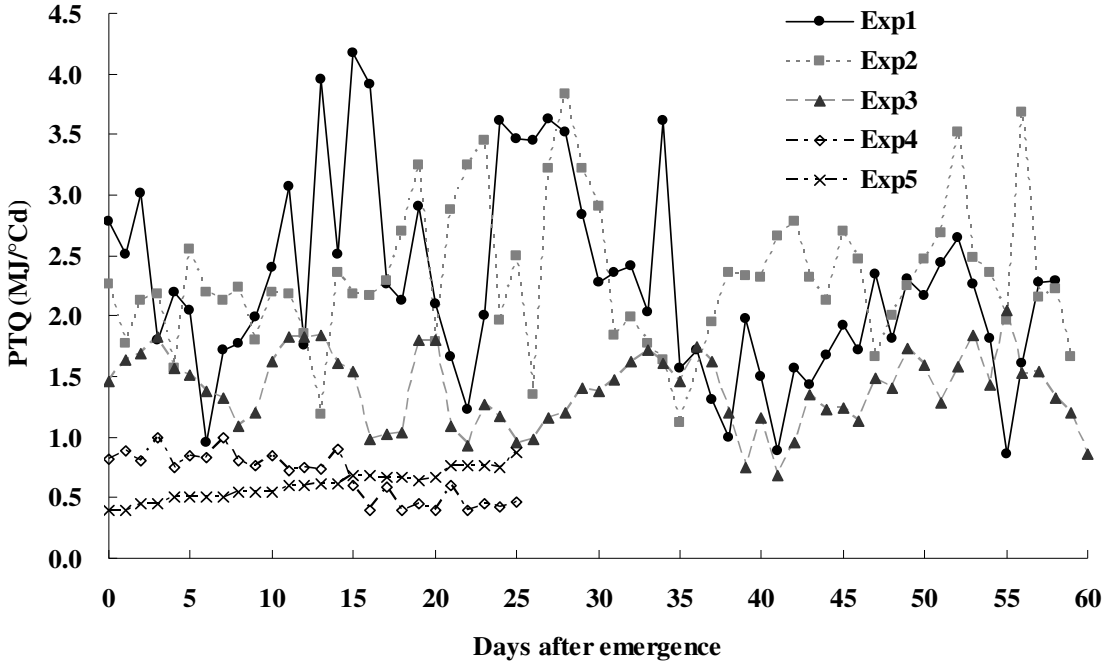


Fig. 1: Daily photo-termal quotient (PTQ) variation during the first 60 days after emergence (DAE) for each experiment (Exp4-5 were conducted for 4 weeks only). Average PTQ was calculated for specific tillering phases (PT: pre-tillering period approximately up to 14 DAE; TEM: Tiller emergence phase between 14 and 30 DAE; TSEN: Tiller survival or senescence period)

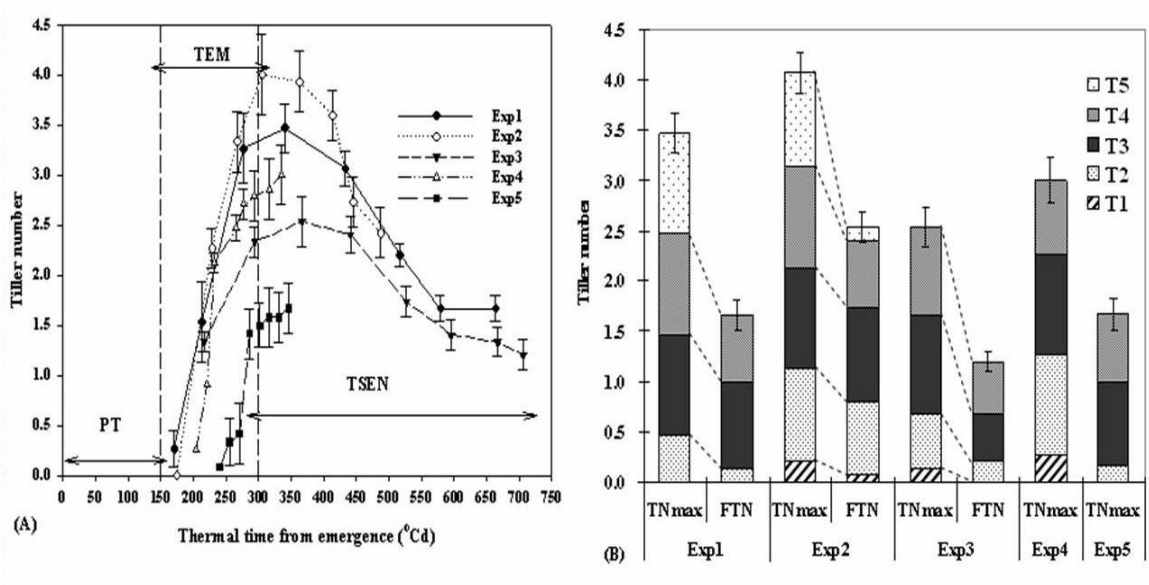


Fig. 2: (A) Tiller number versus thermal time from emergence for the 5 experiments showing the three tillering phases: [PT] pre-tillering between 0 to 150 °Cd after emergence; tiller emergence [TEM] between 150 and 300°Cd approximately; and tiller senescence [TSEN] from the end of [TEM] to flag leaf stage.

(B) Maximum tiller number (TNmax) and fertile tiller number (FTN) and the contribution form each tiller rank (noted T#). In controlled environment experiments (Exps 4, 5), only maximum tiller number was observed.

Tiller appearance and fertility

A common tillering pattern was observed among experiments, with a pre-tillering, tiller emergence, and tiller senescence phase (Fig. 2A). Tiller emergence commenced between 150 and 170°Cd in field experiments, but was slightly and significantly delayed in Exp4 (around 200°Cd) and Exp5 (approximately 250°Cd). Maximum and fertile tiller numbers varied considerably among experiments (Fig 2B), Maximum tiller number was greatest in Exp2 (4.1) and Exp1 (3.5), followed by Exp4, Exp3, and Exp5 (Fig. 2). Therefore, environments with high PTQ tended to produce more tillers than those with a low PTQ.

Environmental differences in TNmax could be related to the frequency of appearance

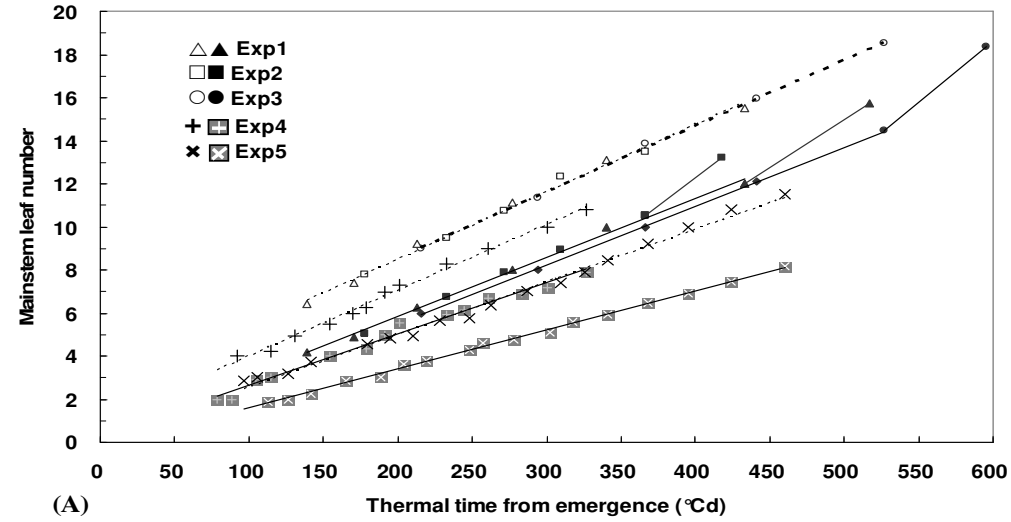
of tillers depending on their rank. Early tiller ranks (T1 and T2) only appeared with a cumulated frequency above 0.5 in high tillering conditions (Exp1, Exp2 and Exp4). T3 and T4 always had the highest frequencies compared to other ranks and T3 had a frequency of 1.0 in all experiments except for Exp5 (where it was still above 0.8). Late tillers (T5 and above) were present only in high-tillering environments (Exp1 and Exp2).

A consistent hierarchy in tillering, both for emergence and fertility frequency, was observed for all environments: T3>T2,T4>T1,T5. Non-fertile tillers emerged slightly later than fertile tillers of the same rank, their leaves stopped appearing during TSEN, and then senesced progressively until anthesis.

Main stem leaf appearance

The number of visible and fully expanded leaves on the main stem increased linearly with thermal time (Fig. 3A). A single linear regression fitted data on tip appearance up to the flag leaf, for all field experiments, resulting in a tip phyllochron of 27.5°Cd (Fig 3A). For leaf ligule appearance, a broken linear regression fitted data from all field experiments, with a breakpoint around the ligule appearance of the largest leaf. This allowed for the more rapid appearance of the last three to four leaves before flag leaf and resulted in ligule phyllochrons of 34.5°Cd and 18°Cd for the two segments respectively in the field experiments. Both ligule and tip phyllochrons were significantly greater in the controlled environment experiments, (Fig. 3A), with a tip phyllochron of 31°Cd (Exp4) and 37°Cd (Exp5). However, the main difference from the field results was observed for ligule phyllochron, which was 40°Cd in Exp4 and over 54°Cd in Exp5. Because of the differences in tip and ligule phyllochron, leaves on each axis had a rank-dependent leaf expansion duration (LED) (Fig. 3A and Fig. 4), increasing with leaf rank up to the largest leaf and then decreasing rapidly until flag leaf, due to the high ligule appearance rate of the upper leaves. The high LED in controlled

environment compared to field conditions was largely related to the slower ligule appearance rate.



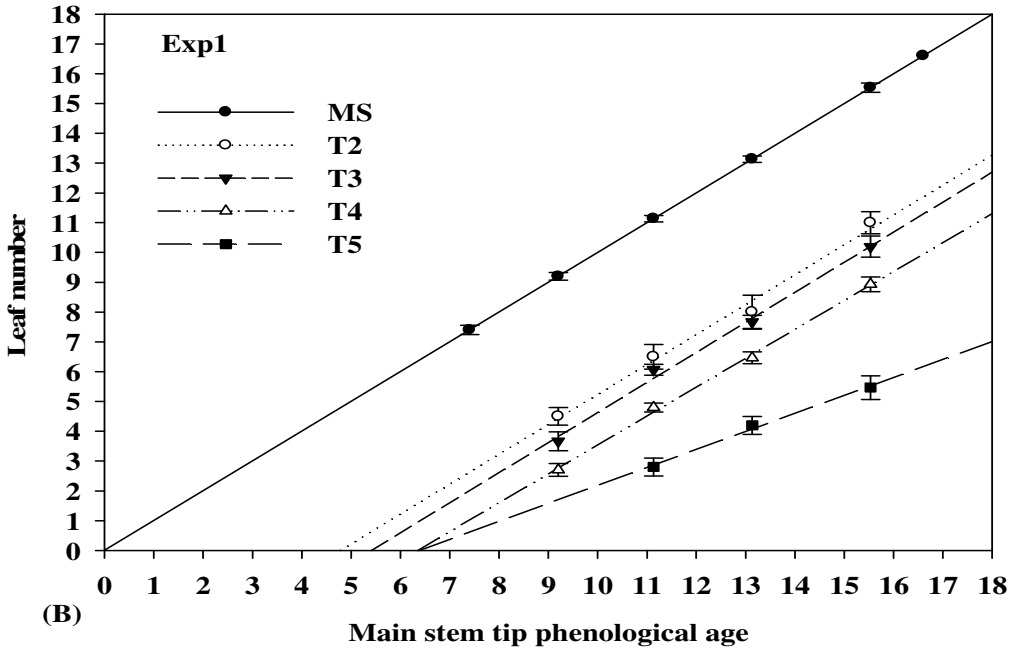


Fig. 3: **(A)** MS leaf tip (open symbols) and ligule (filled symbols) number versus thermal time from seedling emergence in the three field experiments (up to flag leaf) and controlled environment experiments (up to MS L10).

(B) Tiller (T2 to T5) coordination with main stem development in a high tillering experiment (Exp1). For T2 to T4, fertile tillers and non fertile tillers were considered but for T5, there were only non fertile tillers.

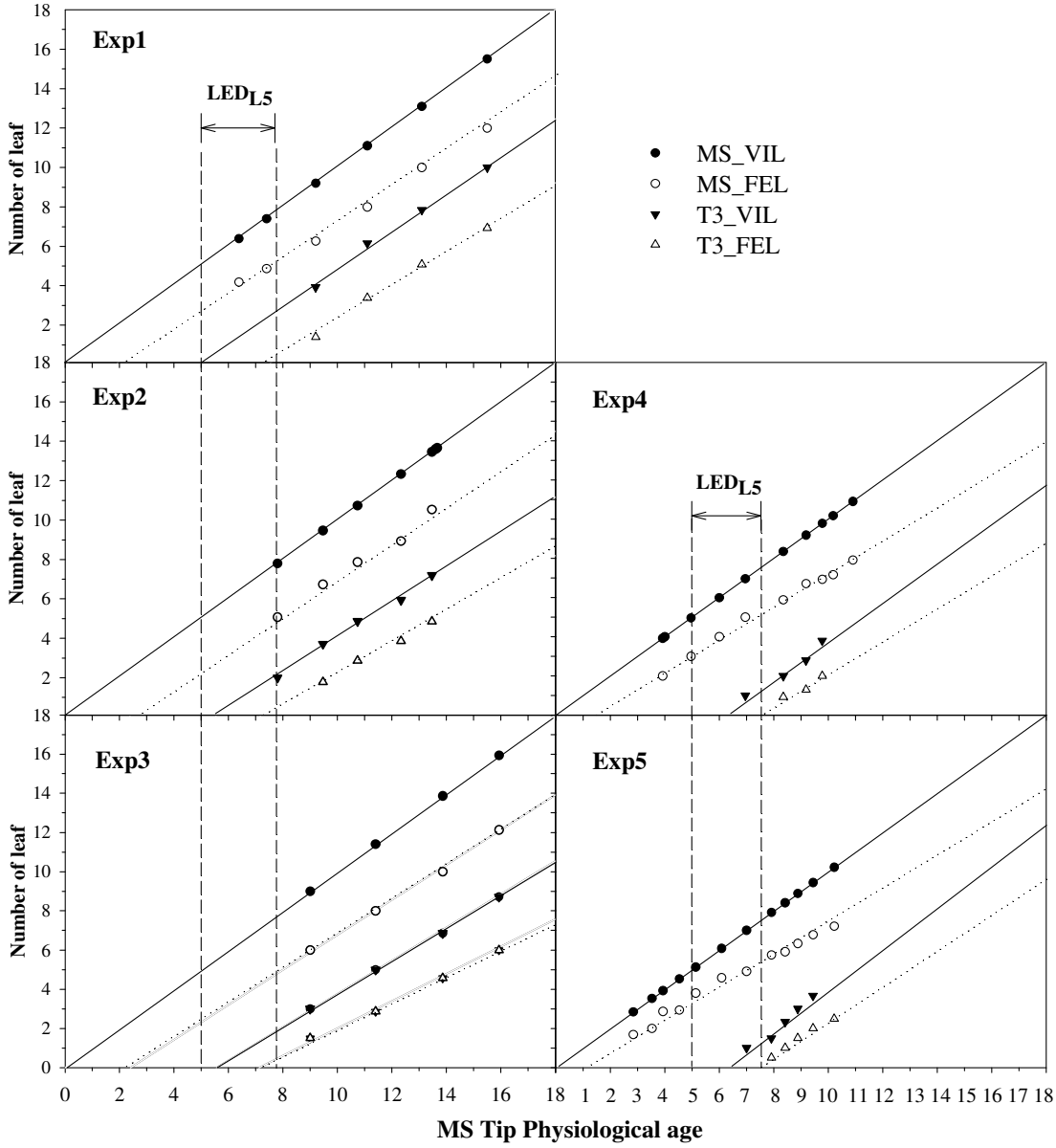


Fig. 4: Synchrony of main stem (MS) and fertile tiller 3 (T3) leaf appearance and ligulation (VIL: visible leaf; FEL: fully expanded leaf). Solid lines represent linear regressions of leaf tip appearance rate and dashed lines represent linear regressions of leaf ligule appearance rate. Standard errors under 0.2 are not represented. Leaf expansion duration of L5 (LED_{L5}) is represented for each experiment.

Coordination between main stem and tillers

Leaf tip and ligule appearance rates of fertile tillers were similar to those of the main stem (Fig. 4). In addition, the phenological age (in terms of leaf tip number) at which a tiller of a particular rank emerged (represented by the intercept with the x-axis of the linear regression of tiller leaf tip number on main stem leaf tip number), was relatively constant across experiments. T3 emerged during a narrow window between the tip appearance of main stem L5 and L6 (field experiments) and main stem L6 and L7 (controlled environment experiments) (Fig. 4). A similar tight relationship between tiller appearance and main stem development was observed for productive tillers of the other tiller ranks. Consequently, tiller appearance was highly coordinated with main stem leaf appearance and each tiller rank had a narrow window for potential appearance: T2 emerged between main stem L4 and L5 tip appearance, T3 between main stem L5 and L6 tip appearance and T4 between main stem L6 and L7 tip appearance. The late onset of tillering in Exp5 compared to Exp4 was therefore a consequence of the low number of lower-ranked tillers. Tiller emergence ceased when around 8-9 leaves had fully expanded and crop LAI of approximately 0.5-0.7 was reached (Fig. 5).

Consistent with this coordination between tiller appearance and main stem leaf appearance, final leaf number declined with successive tillers, such that T4 final leaf number was one less than T3 and two less than T2 (Fig. 3B). This was consistent across all experiments.

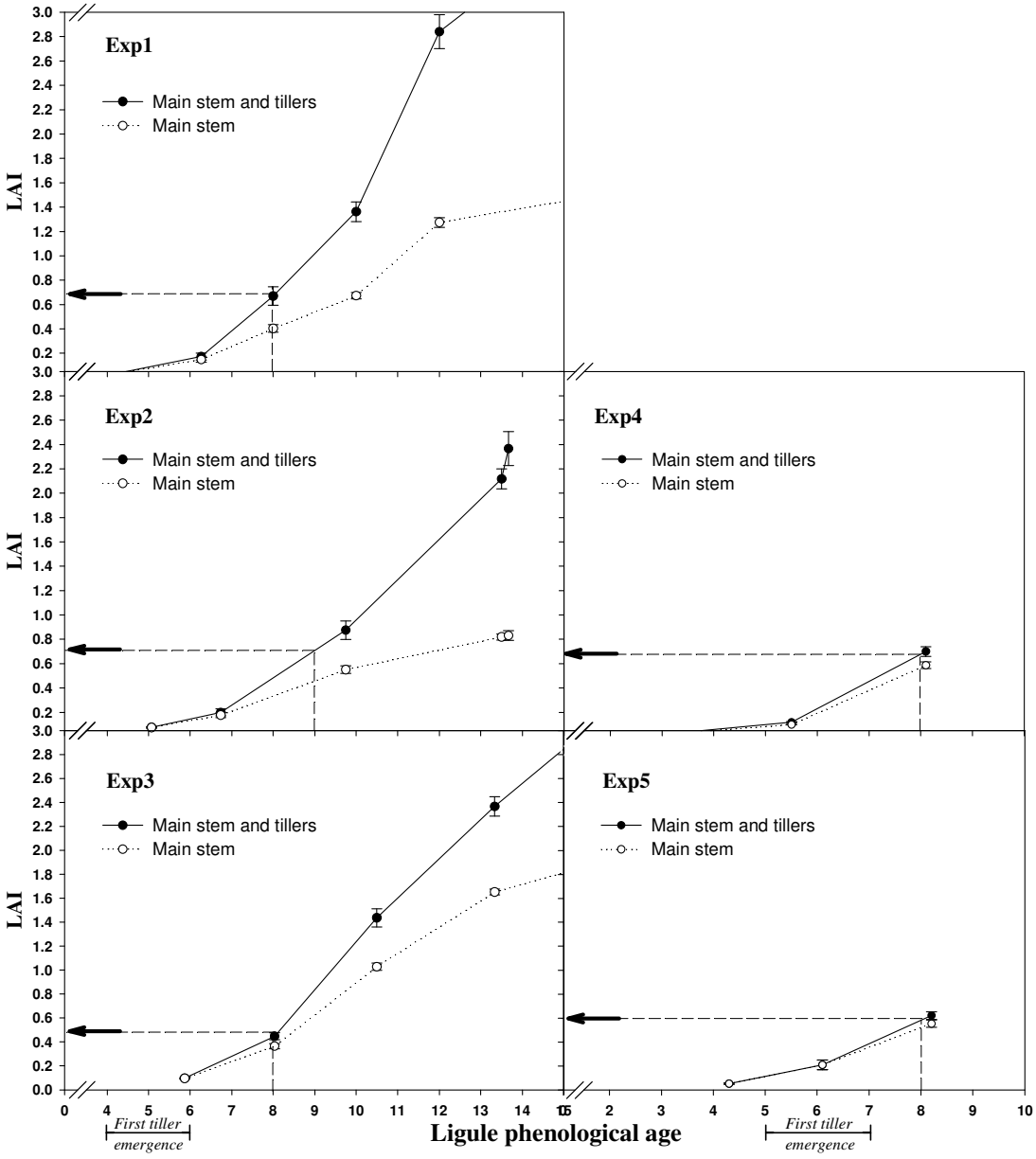


Fig. 5: LAI development at individual plant level for main stem and whole plant during early growth in all experiments. Intervals on ligule phenological age axes indicate corresponding first tiller emergence window in field (left) or controlled environments (right). Horizontal arrows indicate approximate LAI at cessation of tillering for each experiment.

Leaf size

From seedling emergence to the end of TEM phase, TPLA was predominantly determined by main stem leaf area development (Fig. 5 expressed in terms of LAI). Environmental differences in the main stem leaf area profile were observed from L4 (beginning of TEM) onwards (Fig. 7). Between L 4 and L9 (leaves developing during TEM), Exp4 had the largest leaf size while from L6 onwards, Exp2 had the smallest leaves. Main stem leaf area profiles of Exp1 and Exp3 were very similar.

The environmental effects on leaf size were predominantly due to an effect on leaf length, rather than leaf width (Fig. 7B,C). Between L4 and the largest leaf, both the length and the width of successive leaves increased linearly with leaf rank. Therefore, environmental differences in leaf size were a consequence of differences in the slopes of these linear relationships. These slopes, defined here as the Leaf Length Increase rate (LLIR) and the Leaf Width Increase rate (LWIR) thus represent the increase in crop LAI during the tiller period.

Development of a plant S/D framework

RTR as a function of RGR

The considerable variability in tillering behaviour across environments (Fig. 2) was associated with variation in crop growth. In general, RTR increased linearly with RGR, although there was a tendency for controlled environment experiments to have a lower threshold than field experiments (Fig. 6). This supports the hypothesis that carbohydrate availability determines tillering, although the results also suggest that threshold RGR below which no tillers can be produced (the intercept of the regression with the x-axis) differed between field and controlled environments. This indicates that a more specific indicator than RGR is required to capture the different environmental effects operating across the experiments.

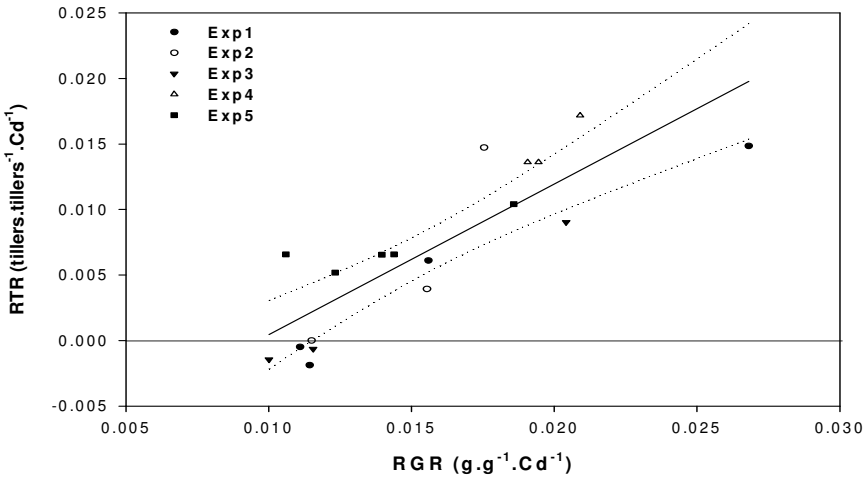


Fig. 6: Relationship between relative tillering rate (RTR) and relative growth rate (RGR) from tiller emergence to tiller cessation across all experiments. The linear regression (RTR = 1.15 x RGR – 0.011, $r^2 = 0.72$) is shown by the solid line, and its 95% confidence interval by the dotted lines.

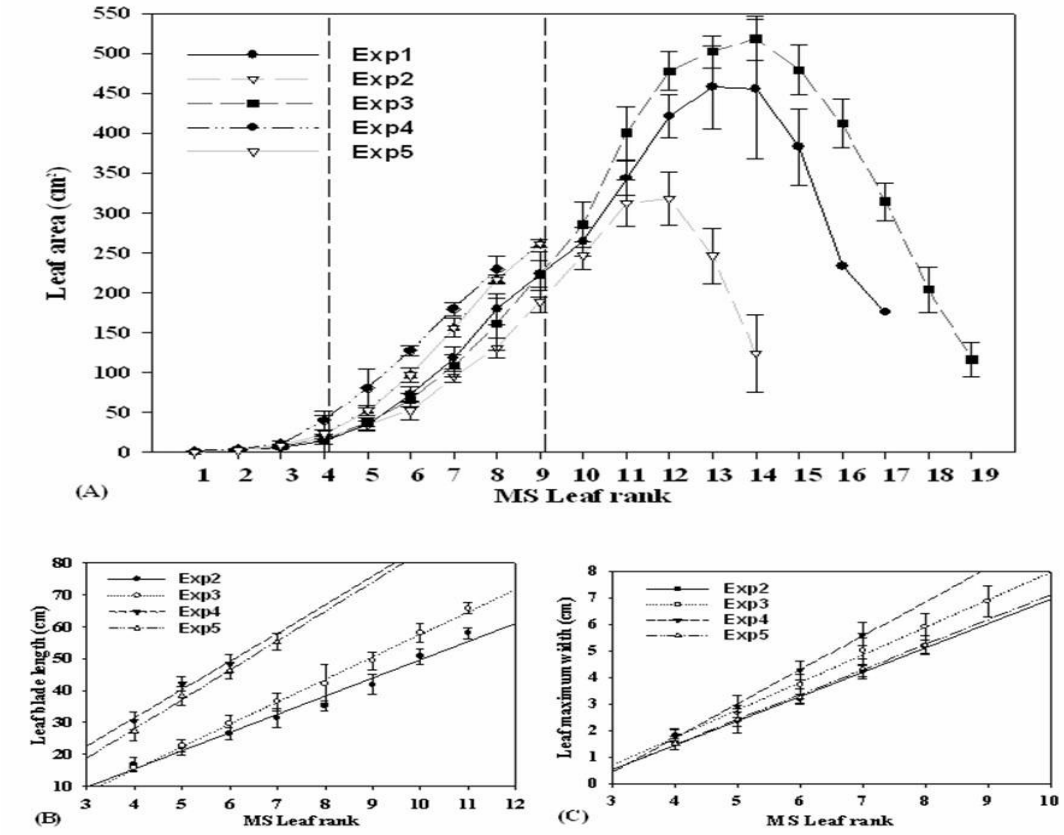


Fig. 7: **(A)** Individual fully expanded leaf area per leaf rank on the main stem (until flag leaf for field experiments and until leaf rank 8 or 9 for controlled environments). Vertical lines represent TEM (tiller emergence phase).

(B) Main stem (MS) leaf blade length and **(C)** width as a function of leaf rank in field and controlled environment experiments (Exp1, identical to Exp3, is not presented).

S/D$_{index}$

The above results are consistent with the hypothesis that internal plant competition for assimilates determines tillering. Therefore, a S/D$_{index}$, based on the concepts described in Eq.4, was developed, to explain the environmental effects on tiller emergence in a supply/demand framework.

At the start of TEM, L5 is expanding (Fig. 4) and its area (LA5) was therefore used to index plant leaf area, and hence light interception, for the duration of the TEM phase (DevPhase) (Eq.4). RAD$_{LED5}$ represents the accumulated radiation per unit of development accumulated during L5 LED. The demand for assimilates was indexed by the product of the L5 LED (TT$_{LED5}$) and LLIR, as the latter was the main factor associated with leaf area increase during the phase. We used the LLIR from L4 to L9 (LLIR$_{L4-9}$), as these represented most of the leaves that were expanding during TEM (Fig. 3 and Fig. 7). The S/D$_{index}$ was thus computed as:

$$S/D_{index} = \frac{RAD_{LED5} \times LA5 \times DevPhase}{TT_{LED5} \times LLIR_{L4-9}} \quad (5)$$

The S/D index was highly correlated with tillering rate, represented by TNmax per unit of development (Fig. 8).

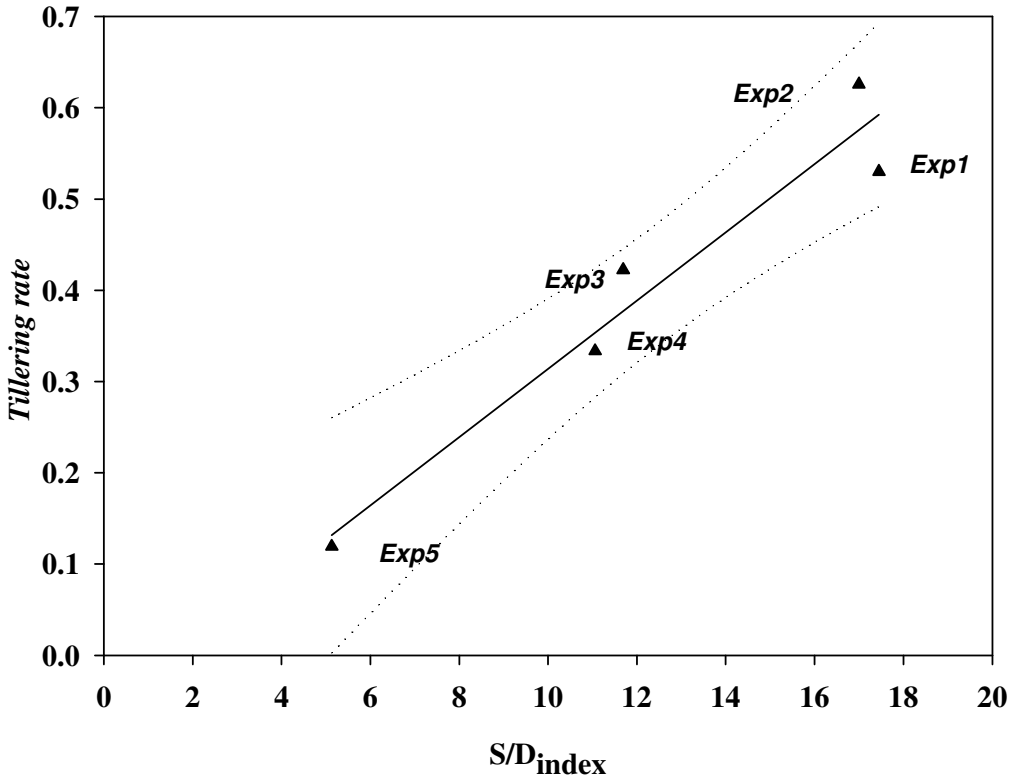


Fig. 8: Tillering rate (TNmax per unit of development) response to S/D_{index}. Variation of supply across environment is estimated as PTQ per unit of development cumulated during MS L5 development x MS leaf 5 area and demand variation of the MS is estimated by its leaf length increase rate (LLIR). Linear regression (***Tillering rate*** **= 0.037 * S/D_{index} – 0.06, r^2 = 0.94**) is represented in plain line, 95% confidence interval in dotted line.

DISCUSSION

In this study, the environmental control of tillering in sorghum via effects on plant carbohydrate S/D status was quantified by growing a representative hybrid (MR Buster) in a range of environments with varying radiation and temperature. The results indicated that tiller appearance was highly synchronised with main stem leaf appearance, with a consistent hierarchy for tillering across environments. The main environmental effect was on the frequency of tiller appearance, in particular of the lower-rank tillers and this explained some of the observed environmental differences in the onset of tiller appearance. These differences were consistent with the hypothesis that internal plant competition determines tillering. A generalised S/D index, that accounted for environmental conditions, plant assimilate supply and demand, and plant development, explained most of the variation in maximum tiller number observed across five experiments.

Tillering is developmentally regulated

Coordination of tillering and leaf appearance

A consistent coordination between main stem leaf development and successive tiller rank emergence was observed across environments (Fig. 3). Each tiller rank had a window of approximately one phyllochron during which it could appear (Fig. 2), although there was a tendency that this window was shifted one leaf rank in controlled environment experiments compared with field experiments. Late emergence of a tiller, towards the end of the appearance window, made a tiller less competitive compared with other tillers, whereas in extreme cases, a particular tiller would not appear at all. This coordination between main shoot leaf appearance and tiller appearance was consistent with previous observations for sorghum (Lafarge and Hammer, 2002), pearl millet (Craufurd and Bidinger, 1988; van Oosterom *et al.*, 2001) and wheat (Porter, 1985; Rickman *et al.*, 1985). At the meristem level, the initiation of cell division and elongation in tiller (N) coincides with the commencement of

cell division in the primordium of Leaf (N+2) (Skinner and Nelson, 1994). This synchrony is consistent with our observation that T3 appeared around the time the tip of main stem L5 became visible. Plant phenological age (either main stem leaf tip or ligule appearance) would therefore be a more robust parameter than thermal time *per se* and consequently, environmental effects on plant phenology can be important factors in explaining tillering dynamics in terms of topological location, appearance and fertility frequency according to growing conditions.

The leaf appearance rate of productive primary tillers was very similar to that of the main stem (Fig. 4), confirming previous results for rice (Tivet *et al.*, 2001) and pearl millet (Craufurd and Bidinger, 1988; van Oosterom *et al.*, 2001). This similarity occurred across experiments, even though ligule phyllochron increased by 6 to 15°Cd in controlled environment experiments, compared to field experiments. The long phyllochron in controlled environment experiments was most likely due to low radiation (<10 $MJ.m^{-2}.day^{-1}$ in Exp5 vs 25 $MJ.m^{-2}$ day^{-1} in field experiments), which may have limited assimilate availability for leaf expansion or invoked shade plant responses (Smith and Whitelam, 1997). Similar effects of low radiation on phyllochron have been reported for maize (Birch *et al.*, 1998).

Cessation of tiller appearance was also highly regulated with main shoot leaf appearance. In general, tillering ceased when the LAI of the crop was 0.6-0.7, except in Exp3 (LAI=0.5). Our results are consistent with the average value of 0.64 reported for MR Buster across a range of densities (Lafarge and Hammer, 2002b). This regulation of cessation of tillering by LAI has been linked to red:far-red ratio of light in the canopy (Casal *et al.*, 1985; Evers *et al.*, 2006).

Hierarchy in tillering

In addition to the coordination of tiller appearance with main stem leaf appearance, there was a consistent hierarchy across tiller ranks in the frequency of both tiller emergence

and fertility frequency, with T3>T2,T4>T1,T5. This hierarchy corroborates the results of Lafarge *et al.* (2002) for the same hybrid, grown at a range of densities in a single environment.

The environmental effect on tillering operated through an effect on the frequency of tiller appearance, in particular for the lower order tillers. Similar effects of environment on the appearance frequency of lower order tillers have been reported for pearl millet (van Oosterom *et al.*, 2001). This effect is consistent with a framework where the environmental effect on tillering is through the timing of the onset of tillering. Because of the coordination between tiller appearance and main stem leaf appearance, a delay in the onset of tillering will predominantly affect the frequency of appearance of lower-order tillers, as these are the tillers that have their window of opportunity for emerging around the onset of tillering. These effects on tiller appearance may reflect differences in assimilate availability for tiller appearance (Bos and Neuteboom, 1998). Jaffuel and Dauzat (2005) observed in rice that the timing and frequency of fertile tillers complied with rules of priority depending on their order, rank and emergence time.

Regulation of tillering through a S/D framework

To account for the effects of environmental conditions and the plant carbohydrate supply/demand balance on tiller appearance, we developed a generalised S/D index (Equation 6) that integrated these effects into the PTQ framework developed by Nix (1976). The index used parameters specific to L5, which was the leaf that was expanding at the emergence of T3. As each tiller had only a narrow window during which it could emerge, the appearance frequency of a tiller rank is a function of the S/D balance during this period. The observation that in most experiments, T3 was always present, suggested that only once L5 was elongating, was the S/D index above the threshold required to initiate a tiller. The S/D index was thus designed to represent the plant carbohydrate S/D status from the onset of tiller appearance.

The main environmental effect on leaf size was through leaf length, rather than width. Similar results have been observed for maize using QTLs (Reymond *et al.*, 2004). The length of successive leaves in Exp1 and Exp3 (Fig. 7) was very similar to that observed by Tanguy and Hammer (2002), suggesting that the difference in leaf length between field and controlled environment experiments was due to long leaves in the controlled environment experiments. This was likely a consequence of growth responses due to the low light intensity (Smith and Whitelam, 1997). A similar effect of radiation levels on leaf length has been observed for maize (Muller *et al.*, 2001). Therefore, demand for assimilates, which depends on the increase in LAI, was represented in our S/D index by the rate of increase of the length of successive leaves (LLIR).

Despite the huge environmental variability (represented by PTQ) and its effect on leaf size, the S/D index provided a unifying framework that could explain the environmental effects on tillering in response assimilate availability. This indicates that the framework is robust across environments and gives it a predictive value that could provide the basis to allow dynamic simulation of tillering in crop growth models.

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"What shall we say the kingdom of God is like,
or what parable shall we use to describe it?

It is like a mustard seed,
which is the smallest seed you plant in the ground.

Yet, when planted, it grows and becomes the largest of all garden plants,
with such big branches that the birds of the air can perch in its shade."

CHAPTER III

Regulation of tillering in sorghum: Genotypic effects

Hae Koo Kim [1,2]

Delphine Luquet [2]

Erik van Oosterom [1]

Michael Dingkuhn [2]

Graeme Hammer [1]

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[1] The University of Queensland, School of Land, Crop and Food Sciences, Brisbane QLD 4072, Australia

[2] CIRAD, UPR 59, Avenue Agropolis Lavalette TA A 59/01 34398 Montpellier, France

ABSTRACT

• *Background and Aims* Genetic variation in tillering affects the dynamics of canopy development and, hence, the timing and nature of crop water limitation. The aim of this paper is to develop a robust framework explaining both genotypic and environmental effects on tillering and their interactions as a dynamic consequence of the underlying internal competition for carbohydrate.

• *Methods* Five hybrids, based on BC1F4 inbred lines that were selected for similar phenology and plant height but contrasting tillering, were grown in five experiments that represented a wide range in radiation and temperature conditions and thus in tillering. Data on leaf area dynamics and on biomass accumulation and partitioning were collected at regular intervals. The internal plant competition for carbohydrate to assess tillering potential was estimated with a supply demand (S/D) index.

• *Key Results* The appearance of main stem leaves and specific tiller ranks was highly coordinated across genotypes and environments. The main genotypic difference was the higher appearance frequency of early tiller ranks in the high-tillering hybrids, which was associated with narrower and hence smaller leaves. A generalised index of internal plant competition, estimating plant assimilate supply and demand through leaf morphogenesis characteristics, accounted for most of the observed variation in maximum tiller number across experiments, although genotypic differences in the relationship between the S/D index and tillering rate suggested high-tillering hybrids have a lower S/D threshold at which tillers appear. The impact of S/D on tiller onset was supported by the negative correlation between main culm leaf area and tiller potential.

• *Conclusions* Our results support the hypothesis that genotypic differences in tillering were associated with differences in the carbon supply demand balance, associated with leaf width and differences in the threshold value at which tillers grow out. Incorporation of our framework into crop growth simulation models would provide insights into the complex genotype*management*environment interactions that determine drought adaptation.

Key words: *Sorghum bicolor* L. Moench, carbohydrate supply-demand ratio, dynamic framework, genotype-by-environment interaction, internal plant competition, leaf area development, leaf width, tiller onset

INTRODUCTION

Tillering is generally recognised as one of the most plastic traits affecting accumulation of biomass and ultimately yield in many field crops. Depending on growing conditions and genotype, a wide range in tiller number is observed in high tillering cereals such as barley (Aspinall, 1961; Canell, 1969), wheat (Bos and Neuteboom, 1998a; Friend, 1965; Kasperbauer and Karlen, 1986; Wilson and Swanson, 1962), rice (Honda and Okajima, 1970) or pearl millet (Rai *et al.*, 1999; van Oosterom *et al.*, 2001) as well as lower tillering cereals, such as or sorghum (Bruns and Horrocks, 1984). Genetic variation in tillering affects the dynamics of canopy development and, hence, the timing and nature of crop water limitation (Hammer, 2006). Simulation studies in sorghum (Hammer *et al.*, 1996) indicated significant yield advantage of high tillering types in high-yielding seasons when water was plentiful, whereas such types incurred a significant disadvantage in lower-yielding water-limited circumstances. Hence, the selection of the best genotype or the most appropriate ideotype (Donald, 1968) is confounded by genotype-by-environment (GE) interactions.

This GE interaction can be extended to the role of tillering across species in different breeding strategies of modern cereals (Doust, 2007). Indeed, in high-yielding circumstances for crops like rice or wheat, one of the most critical characteristics of successful high-yielding varieties was semi-dwarf plant types with high tillering ability (Yoshida, 1972). Conversely, Donald (1968) proposed that a uniculm plant, even for high tillering species, could be more appropriate than freely tillering varieties under poorer growing conditions (Islam and Sedgley, 1981). The presence of non fertile tillers reduces grain yield in water-limiting environments (Jones and Kirby, 1977; Winward *et al.*, 1983) via ineffectual water use. Duggan *et al.* (2005) confirmed this recently by showing that wheat cultivars with a gene for tiller inhibition performed better than the standard tillering cultivars under terminal drought.

As tillering is not simulated dynamically in most existing crop models the underpinning GE drivers of variability in tillering are not captured. Either the crop is

considered as a uniculm plant (Birch *et al.*, 1990), or main culms and tillers are treated similarly (Maas, 1993, Heiniger *et al.*, 1997; Rosenthal *et al.*, 1989), or tillering must be input (Hammer and Muchow, 1994). A sound understanding of the genetic and physiological bases underlying GE for tillering is not yet integrated in current models. While it is known that variation in tillering is highly heritable (Jordan, pers. comm.), the physiological basis of this heritability is not well understood. Dissecting variation in tillering into basic component traits is a means to develop this understanding. Furthermore, if the underlying component traits are easy to measure and show a high level of genetic variation with a low GE interaction, indirect selection may then be feasible (Hammer *et al.*, 2005).

The environmental regulation of tillering in sorghum was characterized in a companion study (Kim *et al.*, 2008). Tillering was found to be regulated by internal competition for resources during the early developmental stages of the plant. A generalised index of internal plant competition that took account of plant assimilate supply and demand (S/D index) explained most of the variation in tiller number across a diverse set of experiments. The main environmental effect was on the frequency of tiller appearance, in particular of the lower rank tillers.

To explore the genetic regulation of tillering in sorghum as a trait of interest for breeding (van Oosterom *et al.*, 2006), while maintaining the driving concept of plant internal competition for resources (Lauer and Simmons, 1985; Pieters *et al.*, 2001), the objective of this study was to identify the physiological basis of key genotypic differences in tillering. A set of selected sorghum hybrids known to differ in tillering was grown over a wide range of environmental conditions for this purpose. The framework developed for environmental regulation of tillering in the companion study (Kim *et al.*, 2008) was used as a basis to explore the nature of generic regulation. The intent was to develop a robust framework explaining both G and E effects on tillering and their interactions as a dynamic consequence of the underlying internal competition for carbohydrate.

MATERIALS AND METHODS

Experimental details

Plant material

The experiments included five hybrids varying in tillering obtained by crossing BC1F4 inbred lines (R999218, R999066, R999100, R999197 and R999017) from an advanced backcross (31945-2-2//31945-2-2/*S. arundinaceum*) onto an elite male sterile parent (A23171). The recurrent parent 31945-2-2 is lower tillering, and has been developed by the sorghum breeding program of the Queensland Department of Primary Industries & Fisheries (QDPI&F), whereas *Sorghum arundinaceum* (*S. bicolor* ssp. *arundinaceum*) is an African wild type sorghum with a high tillering ability. The five inbred lines were selected for contrasting tillering behaviour but similar anthesis date and plant height at maturity, based on an experiment that included the entire mapping population (over 200 lines). The selections included hybrids based on two low tillering lines (A23171/R999218 (hybrid 1) and A23171/R999066 (hybrid 2)) and three high tillering lines (A23171/R999100 (hybrid 4), A23171/R999197 (hybrid 5) and A23171/R999017 (hybrid 6). The experiments also included the hybrid based on the recurrent parent (A23171/31945-2-2, hybrid 3) as a low tillering check, and the commercial hybrid MR Buster as a high tillering check (Kim *et al.*, 2008). All hybrids were included in all experiments, with the exception of hybrid 3, which was not included in Exp1, and hybrid 6, which was not included in the controlled environment experiments (Exp4 and Exp5, see below).

Experimental set-up

The experiments presented here included three field (Exp1, Exp2, and Exp3) and two controlled environment (Exp4 and Exp5) experiments, which were described in detail by Kim *et al*. (2008). Here we provide only an overview of these experiments.

The field experiments were conducted at Warwick (28º12’S, 152º5’E, 462m) and

Gatton (27°34' S, 152°18' E, 94m) in south east Queensland, Australia. Exp1 was sown at Warwick on 25 October 2004 and was characterised by low temperature and high daily radiation levels. Exp2 was sown on 2 March 2005 at Warwick and experienced medium temperatures during tillering with high radiation levels. Exp3 was sown on 16 January 2006 at Gatton and experienced high temperatures during tillering and high daily radiation levels. The contrasting temperature/radiation regimes ensured a range in tiller numbers across experiments.

Each field experiment was laid out as a randomised complete block design with three replicates. Plot size was 4 rows of 15 meters, with a row spacing of 1 meter. The 2 central rows were used for data collection. Plants were thinned to a density of 5 plants m^{-2} around two weeks after emergence. All experiments were well-watered and well-fertilised. Exp1 and Exp2 stopped at anthesis, whereas Exp3 was conducted till physiological maturity.

Controlled environment experiments were conducted in a glasshouse (Exp4) and phytotron (Exp5) at CIRAD research center (43°38'N, 3°52'E, 46 m) in Montpellier France, in the summer of 2005. Both experiments were laid out as a randomised block design with three replications per block. Seeds were germinated for one day at 30ºC in an illuminated culture chamber, and subsequently transplanted in drained 1-L pots containing fertilised soil. Pots were watered at least once daily to field capacity with a culture solution (pH 5.5) containing all essential micro-nutrients. In Exp4, natural sunlight was supplemented with halogen lamps during cloudy days to maintain at least 300 $\mu mol.m^{-2}.s^{-1}$ of photosynthetic active radiation (PAR). Temperature was controlled to maintain approximately 20ºC during night and a cooling system was used when temperature exceeded 35ºC. In Exp2, air temperature was maintained at 28ºC/22ºC (day/night), relative air humidity was between 60% and 80% (day/night), and PAR was supplied with halogen lamps during a 13h photoperiod. Experiments were conducted until the end of the tillering stage.

Plant measurements

Phenological and leaf size observations

Seedling percent of emergence was scored daily on 2m per row in each plot until complete emergence in field experiments and on all individual plants in controlled environment experiments. In the field experiments, five consecutive plants in one of the two middle rows of each plot were tagged for weekly counting of the number of visible, fully expanded, and senesced leaves for each axis (main shoot and tillers). A leaf was counted as visible if its leaf tip was visible inside the whorl, fully expanded if its ligule was located above the ligule of the previous leaf, and senesced if less than 50% of its leaf area was green. Tillers were labelled according to leaf axil of origin (e.g. T3 as tiller which appeared from the axil of leaf 3). Tillering dynamics was characterised as detailed by Kim, *et al*. (2008). Anthesis was scored on the same five plants. In Exp3, which was grown until maturity, main stem panicles of ten plants per plot were screened for the presence of black layer on individual grains (physiological maturity).

Individual leaf size in Exp1 was measured for each plot with a planimeter (Delta-T) on fully expanded leaves from three plants that had been destructively sampled at three different growth stages (6 and 12 fully expanded leaves and flag leaf on the main stem). In other field and controlled environment experiments, the fully expanded leaf area of each leaf of each culm was estimated by non-destructive measurements of leaf blade length and maximal width on the five plants in each block in Exp2 and Exp3 and on one plant per repetition in Exp4 and Exp5. Blade length was measured from the ligule to the tip of a leaf. Blade width was measured at its maximal point which was approximately at mid blade length. Leaf blade area (LA) was then calculated by multiplying length and width by a shape coefficient, which ranged from 0.85 (main stem L1) to 0.75 (L2) to 0.69 (all other leaves). These coefficients were kept fixed with culm origin and environmental variations (Kim *et al.*, 2008).

Destructive biomass samples

Biomass accumulation in the field was determined by destructive harvesting on an area of 2 m^2 (10 plants) at approximately weekly intervals, starting before tiller appearance for the first sample and finishing around anthesis (Exp1, Exp2) or maturity (Exp3) for the last sample. A total of five (Exp2) or six (Exp1, Exp3) samples were taken per plot. Plants were cut at ground level and transported to a laboratory, where they were separated into main shoots and tillers, with tillers separated by rank (T1 through to T5). Identification of tiller rank was facilitated by prior marking in the field at the time of tiller appearance early in the season. The number of main shoots (plants) and tillers (by rank) was recorded. Each sample (culm) was separated into green leaves, dead leaves, stems (including leaf sheaths), and panicles (only those above the flag leaf ligule were considered). Green leaves were used to measure green leaf area using a planimeter. All samples were dried at 80ºC for at least 5 days in a fan forced oven before recording dry mass. The data were used to calculate leaf area index (LAI), leaf area ratio (LAR, leaf area divided by total shoot biomass, $m^2.g^{-1}$), specific leaf area (SLA, green leaf area divided by green leaf mass, $cm^2.g^{-1}$), relative growth rate (RGR) and relative tillering rate (RTR) as detailed by Kim *et al.* (2008).

In the controlled environment experiments, two (Exp4) or three (Exp5) samples were taken for shoot and root dry mass and plant leaf area prior to and during tiller emergence. Dry matter samples were divided into organs and axes, similar to the approach used for the field experiments, except that leaf sheaths were separated from the stem and roots were also sampled to determine partitioning between root and shoot (R/S ratio). Plants were sampled early in the morning to minimize variation in dry mass caused by accumulation of carbohydrate reserves. After each destructive sampling, pots were rearranged to maintain a canopy of five plants per meter (same density as in the field) with border plants.

Data analysis

Thermal time was calculated from hourly data, using a broken linear relationship with cardinal temperatures of 11°C, 30°C, and 42°C for the base, optimum, and maximum temperature (Hammer *et al.*, 1993).

Statistical analysis of data was performed using standard analysis of variance procedures using R (R Development Core Team, 2007). Combined analyses of variance across experiments were performed using the AOV procedure, after verifying the homogeneity of variance errors. Locations and replications (blocks) were considered random factors and the remaining effects fixed. Comparisons between lines within an experiment were performed using Tukey's HSD method.

The equations used to calculate the photo-thermal quotient (PTQ), relative tillering rate (RTR) and relative growth rate (RGR) are described by Kim *et al.* (2008), who developed a plant carbon supply /demand (S/D) index for the environmental effect on tillering:

$$S/D_{index} = \frac{RAD_{LED5} \times LA5 \times DevPhase}{TT_{LED5} \times \Delta LA} \qquad [1]$$

With RAD_{LED5} the incident daily global radiation in $MJ.m^{-2}.day^{-1}$ during the expansion of main stem Leaf5 (L5), *LA5* the size of L5 (the last fully expanded leaf at the start of tillering); *DevPhase* the leaf expansion duration of L5; TT_{LED5} the thermal time accumulation during expansion of L5; and ΔLA the increase in leaf area, which was indexed by $LLIR_{4\text{-}9}$, the rate of increase in length of successive leaves. The use of the size of the last fully expanded leaf in this framework reflects the assumption that early in the season all leaf area was intercepting light (Lafarge and Hammer, 2002). Eq. 1 was used as a basis for the development of a S/D index that incorporated the effects of genotype as well as environment on tillering.

Table 1: Summary of the growing conditions for each experiment in terms of location, sowing time, photoperiod, average climate conditions and photo-thermal quotient (PTQ) during distinctive vegetative stage (PT: pre-tillering period; TEM: Tiller emergence phase; TSEN: Tiller survival or senescence phase).

		Exp1	Exp2	Exp3	Exp4	Exp5
Experiment site		Warwick, Field	Warwick, Field	Gatton, Field	Montpellier Glasshouse	Montpellier Phytotron
Location (lat., long., alt.)		28°12'S, 152°5'E, 462m		27°34'S, 152°18'E, 94m	Montpellier, France 43°38'N, 3°52'E, 46m	
Sowing date		25 Oct. 2004	2 Mar. 2005	16 Jan. 2006	31 Aug. 2006	2 Oct. 2006
Photoperiod at sowing		13h02	12h37	13h31	13h13	13h00
Max daily radiation ($MJ.m^{-2}.day^{-1}$)		22.9	21.0	20.3	15.0	9.0
Average daily temperature (°C)		20.5	20.9	26.7	29.9	25.0
Average daily humidity (%)		58.0	60.2	65.6	63.2	70.0
PTQ ($MJ.m^{-2}.°Cd^{-1}$)	**[PT]**	2.4	2.1	1.5	0.9	0.5
	[TEM]	3.3	2.6	1.3	0.5	0.7
	[TSEN]	1.9	2.3	1.4		

Table 2: Maximum tiller number (TNmax) and total appearance for each tiller rank (T_T#), and total fertile tiller number per plant (FTN) and fertility frequency for each tiller rank (F_T#) for each genotype (G) across all environments (E) or each E across all G. G, E and GxE interaction significance are represented. Significance levels: "NS" not significant (p>0.1); (.) at p<0.1; (*) at p<0.05, (**); at p<0.01 and (***) at p<0.001.

	T_T1	T_T2	T_T3	T_T4	T_T5	TNmax	F_T1	F_T2	F_T3	F_T4	F_T5	FTN
Hybrid 1	0.01	0.26	0.58	0.33	0.00	1.19	0.00	0.09	0.42	0.09	0.00	0.60
Hybrid 2	0.03	0.33	0.69	0.43	0.00	1.49	0.00	0.11	0.56	0.38	0.00	1.04
Hybrid 3	0.04	0.25	0.58	0.23	0.00	1.09	0.00	0.13	0.40	0.20	0.00	0.73
Hybrid 4	0.14	0.69	0.89	0.49	0.03	2.24	0.04	0.40	0.84	0.47	0.02	1.78
Hybrid 5	0.01	0.50	0.78	0.44	0.03	1.76	0.00	0.38	0.80	0.58	0.00	1.76
Hybrid 6	0.00	0.47	0.83	0.60	0.00	1.90	0.00	0.37	0.70	0.27	0.00	1.33
Exp1	0.01	0.41	0.99	0.98	0.19	2.58	0.00	0.20	0.84	0.49	0.00	1.53
Exp2	0.09	0.60	0.92	0.87	0.18	2.66	0.03	0.40	0.83	0.56	0.03	1.86
Exp3	0.05	0.36	0.56	0.24	0.00	1.21	0.00	0.20	0.32	0.15	0.00	0.68
Exp4	0.10	0.65	0.65	0.12	0.00	1.52						
Exp5	0.02	0.09	0.70	0.19	0.00	1.00						
G	***	***	***	***	***	***	*	***	***	***	*	***
E	*	***	***	***	***	***	NS	***	***	***	NS	***
GxE	NS	NS	**	***	NS	.	NS	*	NS	***	NS	*

RESULTS

Environmental characterisation

The different sowing dates and locations generated environmental variation in daily radiation, temperature, and their ratio, as well as in photoperiod (Tab. 1). The average daily incident radiation consistently exceeded 20 $MJ.day^{-1}$ in the field experiments, but was no more than 15 $MJ.day^{-1}$ in the glasshouse experiment and below 10 $MJ.day^{-1}$ at last harvest in the phytotron. Daily temperatures on average were lowest in Exp1 and Exp2, highest in Exp4, and intermediate in Exp5 and Exp3. As a consequence, the photo-thermal quotient (PTQ, Tab. 1) mostly exceeded 2 $MJ.m^{-2}.°Cd^{-1}$ in the first two field experiments (Exp1 and Exp2), but was below 1 $MJ.m^{-2}.°Cd^{-1}$ in the controlled environments (Exp4 and Exp5), with Exp3 intermediate.

Genotypic differences in frequency of early tiller ranks

All hybrids had three distinctive tillering phases (Fig. 1): (i) a phase prior to tiller appearance [PT], which varied from 150 to 250ºCd (thermal time from emergence), depending on genotype and environment, (ii) a relatively short tiller appearance phase [TEM] (no more than 150 ºCd after first tiller emergence), and (iii) a phase during which a fraction of the tillers progresses towards anthesis (fertile tillers) and the other fraction ceases to develop and ultimately senesces [TSEN].

The maximum total tiller number (TNmax) and final fertile tiller number (FTN) showed significant ($P<0.001$) G and E effects (Table 2). However, the rankings of the hybrids were generally constant across experiments. In each experiment, the hybrids could therefore be classified into a low tillering (LT, hybrids 1-3) and a high tillering group (HT, hybrids 4-6), based on TNmax. The two groups were statistically different according to Tukey's HSD method. In general, the grouping was consistent across field experiments, except for hybrids 2

and 5 in Exp4 (Fig. 1). Across all experiments, hybrids 4 and 6 had the highest TNmax and hybrids 1 and 3 (recurrent parent hybrid) the lowest TNmax. As a consequence, the GE interaction in terms of total tiller number was small compared to the G and E main effects (Tab. 2).

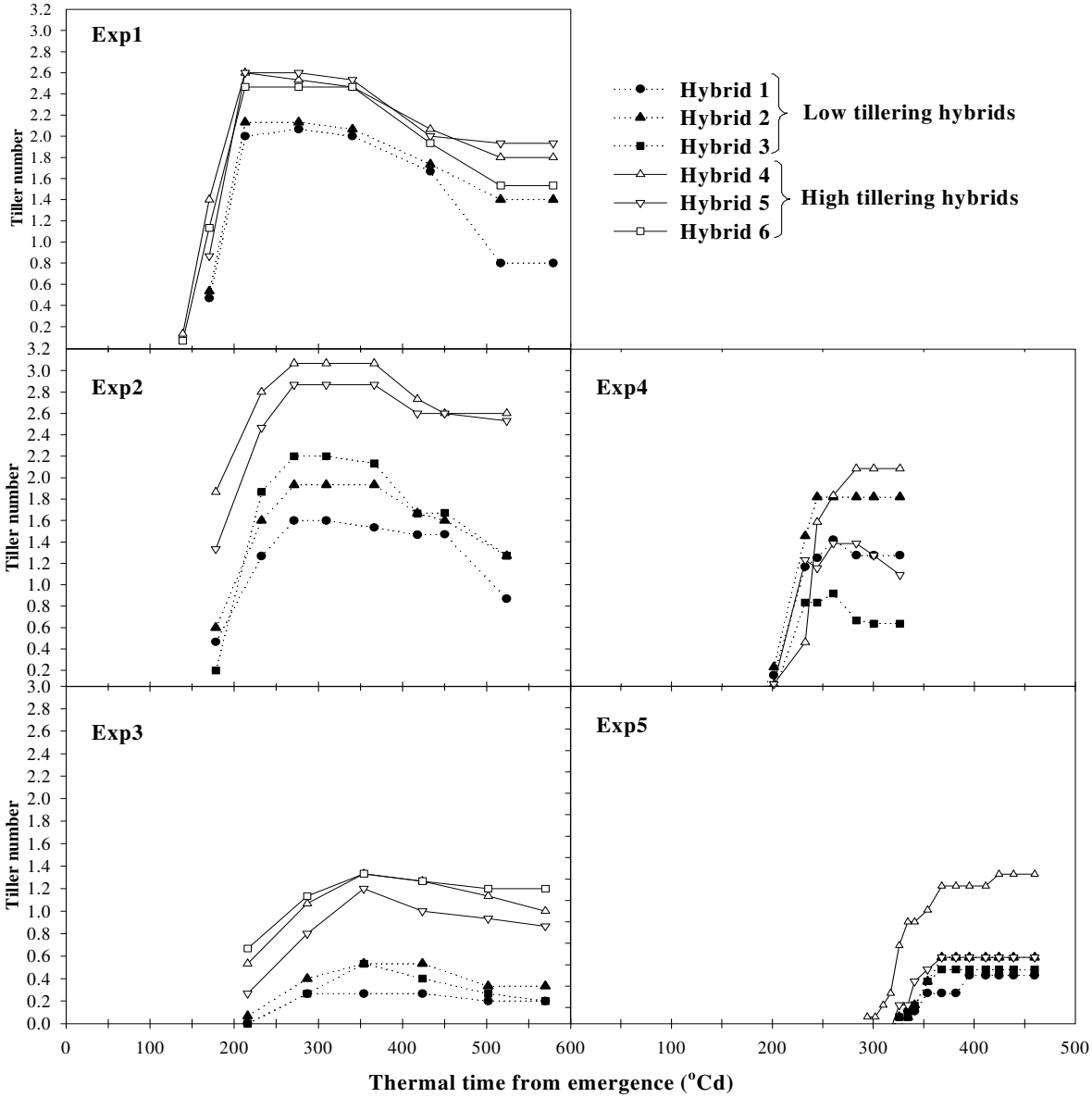

Figure 1: Tillering dynamics of each hybrid in field (Exp1-3) and controlled environment experiments (Exp4-5). Low tillering hybrids (hybrids 1-3) are depicted by filled symbols and dotted lines and high tillering hybrids (hybrids 4-6) by empty symbols and plain lines. Exp1 and Exp2 are high tillering environments, Exp3 and Exp5 are low tillering whereas Exp4 is intermediate (s.e.m below 0.2 are not represented).

Individual tiller ranks, by contrast, displayed varying GE interactions for both the appearance frequency and the fertility frequency: there was a higher GE effect for the appearance frequency of T3, T4 and T5, but not for the appearance frequency of early tiller ranks (T1 and T2). The number of tillers that appeared but ceased growth prior to full flag leaf appearance was on average very similar across the three field experiments for the two groups of hybrids (0.73 for LT, 0.62 for HT). However, LT hybrids tended to have a higher number of T3 and T4 that ceased growing, whereas the number of non-productive T1, T2, and T5 tillers was higher for HT hybrids (Tab. 2).

In general, the onset of tillering was slightly later in the LT hybrids than in the HT hybrids (Fig. 1). This difference was associated with a lower frequency of occurrence of lower order tillers (T1 and T2) in LT hybrids compared with HT hybrids, rather than to a delayed appearance of tiller of a specific rank. In Exp1 and Exp2 (two high-tillering experiments), the difference between HT and LT hybrids in appearance frequency of T2 accounted for 76% (Exp1) and 51% (Exp2) of the difference in TNmax; in the low-tillering Exp3, the difference in appearance frequency of T2 plus T3 accounted for over 80% of the difference in TNmax. By contrast, LT and HT groups did not differ in the timing of appearance of T3, if expressed in terms of tip physiological age either in a high-tillering (Exp1) or in a low-tillering (Exp3) environment (Fig. 2A, B).

Table 3: Main stem (MS) phenology principal characteristics. G (genotypic), E (environment) and GxE interaction significance are indicated. Small letters indicates similar groups according to Tukey's HSD method. Significance levels: "NS" not significant (p>0.1); (.) at p<0.1; (*) at p<0.05; (**) at p<0.01 and (***) at p<0.001.

	MS leaf number	Thermal time to flag leaf	tip phyllochron	lig phyllochron	tip − lig phyllochron
Exp1	15.1[a]	530[a]	27.0[a]	33.8[a]	6.8[a]
Exp2	13.9[b]	480[b]	28.7[a]	35.8[a,b]	7.1[a]
Exp3	17.0[c]	590[c]	29.5[a,b]	36.6[b]	7.1[a]
Exp4			30.4[b]	39.4[c]	9.0[b]
Exp5			39.4[c]	55.4[d]	16.0[c]
G	NS	NS	NS	NS	NS
E	***	***	***	***	***
GxE	NS	NS	NS	NS	NS

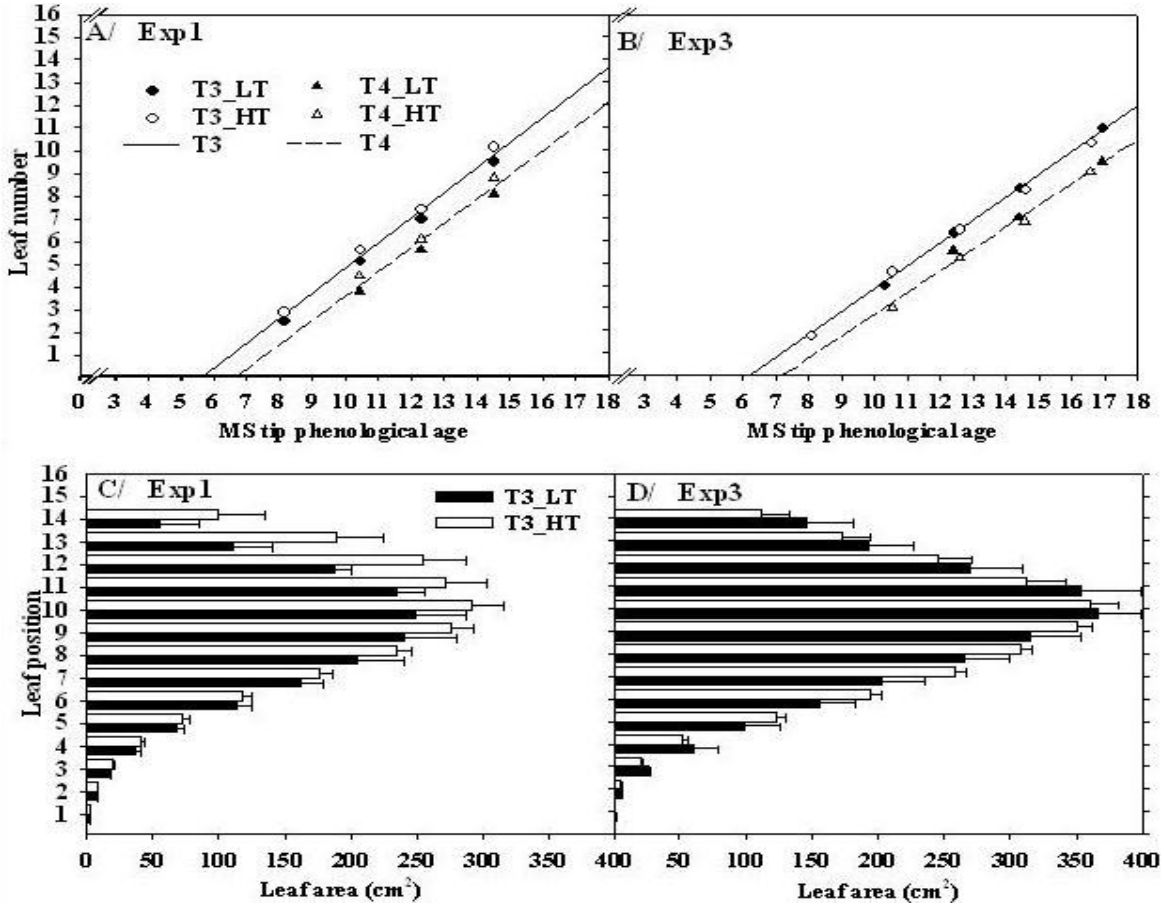

Figure 2: (A, B) Fertile T3 and T4 leaf appearance vs. main stem (MS) tip phenological age in a high (Exp1) and low (Exp3) tillering environment for LT (hybrids 1-3) and HT (hybrids 4-6) groups.

(C, D) Fertile T3 individual leaf area profile for LT (hybrids 1-3) and HT (hybrids 4-6) groups in Exp1 and Exp3.

Phenology and coordination of main stem and fertile tillers.

Main stem phenology was very similar across the hybrids. In none of the experiments did we observe a significant difference in phyllochron (both tip and ligule), or in total leaf number on the main shoot. Consequently, the thermal time form emergence to full flag leaf emergence was also similar across hybrids (Tab. 3).

Field experiments differed little in phyllochron, but had significantly different leaf number, and hence time to flag leaf. The low leaf number in Exp2 was associated with a short day length, whereas the high leaf number in Exp3 was associated with a long day length and high temperature. However, the absence of significant GE interactions for main shoot leaf number or time to flag leaf stage (Tab. 3) indicated that the hybrids responded similarly to environmental cues.

Within an experiment, leaf appearance rates (tip and ligule) on fertile tillers were highly synchronised with main shoot leaf appearance (Fig. 2A,B). T3 appearance occurred at the same phenological age (around main stem L5-L6 tip appearance in field experiments and L6-L7 in controlled environments) for all hybrids within a given experiment, and the phyllochron of fertile tillers was not significantly different from the main shoot. The lower leaf number of tillers compared with main shoots compensated for their later appearance, resulting in a synchronisation of phenology between tillers and main shoot.

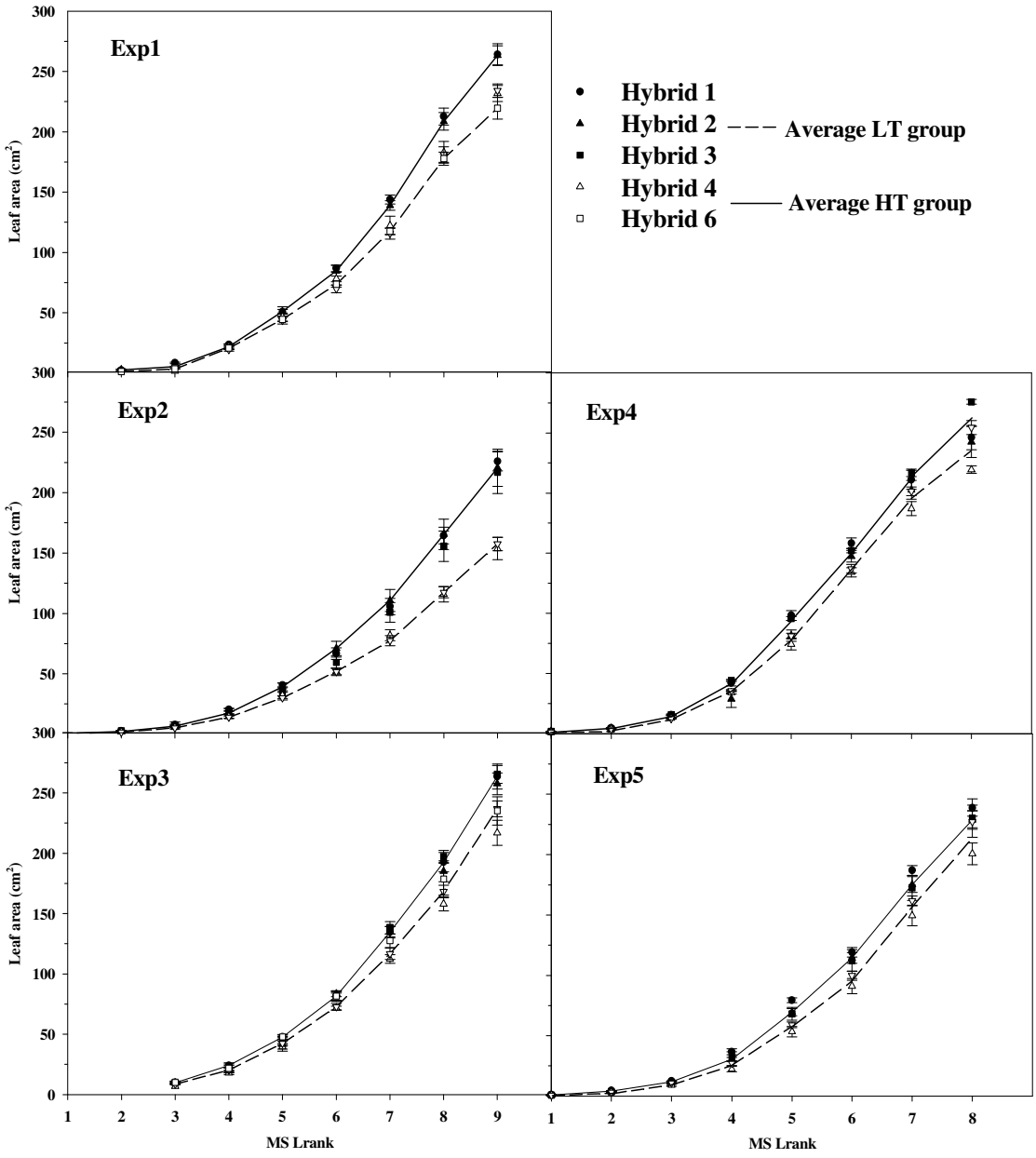

Figure 3: MS individual leaf area profile (from L1 up to L9) in each environment and for corresponding low tillering (LT) or high tillering (HT) hybrids. Solid line represents average of LT hybrids, dotted line average of HT hybrids in field (Exp1-3) and in controlled environments (Exp4-5).

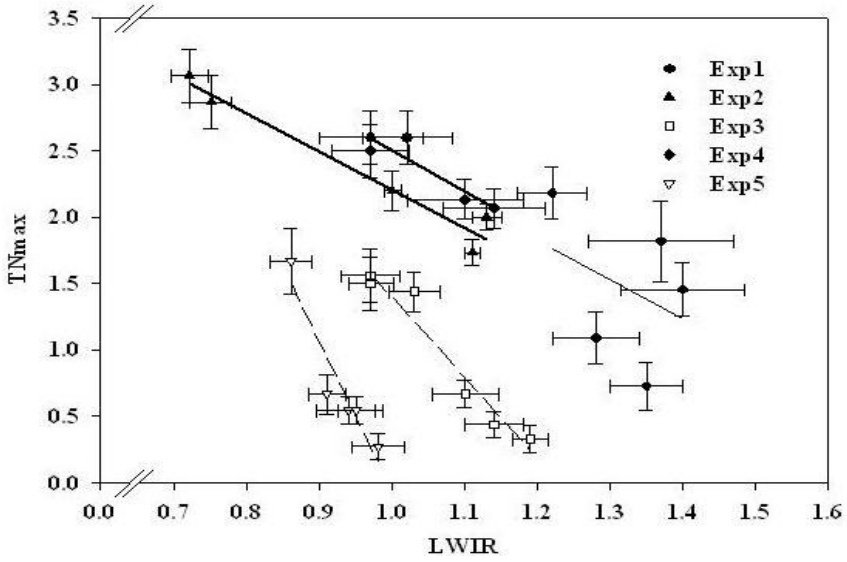

Figure 4: Relationship between leaf width increase between main stem L4 and L9 (LWIR) and maximum tiller number (TNmax) for each hybrid in each respective experiment. Solid lines represent linear regressions of all hybrids in each experiment.

Table 4: Summary of means of LLIR (leaf length increase rate) and LWIR (leaf width increase rate) between main stem L5 to L9 for each environment (E) (except Exp1, which was similar to Exp3) and each genotype (G). Corresponding G, E and GxE interaction significance are indicated. Significance levels: "NS" not significant (p>0.1); (.) at p<0.1; (*)

E	G	LLIR	LWIR
Exp2	Hybrid 1	5.42	1.11
	Hybrid 2	5.47	1.13
	Hybrid 3	5.44	0.84
	Hybrid 4	5.89	0.72
	Hybrid 5	5.92	0.75
Exp3	Hybrid 1	6.68	1.14
	Hybrid 2	6.86	1.10
	Hybrid 3	6.92	1.19
	Hybrid 4	6.47	0.97
	Hybrid 5	6.57	1.03
	Hybrid 6	6.65	0.97
Exp4	Hybrid 1	9.30	1.40
	Hybrid 2	9.56	1.37
	Hybrid 3	10.69	1.28
	Hybrid 4	10.11	1.22
	Hybrid 5	10.85	1.30
Exp5	Hybrid 1	9.18	0.98
	Hybrid 2	9.49	0.94
	Hybrid 3	9.30	0.95
	Hybrid 4	9.79	0.86
	Hybrid 5	10.09	0.91
G		NS	***
E		***	***
GxE		NS	*

Genotypic differences in leaf area dynamics

Individual leaf size on the main shoot differed significantly among hybrids from L5 onwards in field experiments and from L4 onwards in controlled environment experiments (Fig. 3). In particular between L5 and L9, LT hybrids had significantly larger leaf size than HT hybrids and this was predominantly due to wider leaves, rather than longer leaves (Table 4). Both the length and width of successive leaves increased linearly with leaf rank between L4 and L9, but the leaf width increase rate (LWIR) differed significantly between HT and LT

hybrids, whereas the leaf length increase rate (LLIR) was not significantly different between the two groups of hybrids. As a consequence, for each of the environments there was a negative correlation between TNmax and LWIR (Fig. 4).

The larger main stem individual leaf size of LT hybrids, combined with the absence of genotypic differences in main shoot final leaf number (Table 3) and leaf senescence (data not shown), resulted in LT hybrids consistently having a higher main stem LAI than HT hybrids (Fig. 5). In Exp1 and Exp2, this difference in main shoot leaf area was compensated by a difference in tiller leaf area, resulting in very similar LAI at crop level for the LT and HT hybrids. In Exp3, by contrast, the difference in tiller leaf area between the two groups was much larger, resulting in a significantly higher LAI at flag leaf stage for HT than LT hybrids (Fig. 5).

For fertile tillers, there was no significant difference between LT and HT hybrids in individual leaf size at a specific rank until the largest leaf was reached (Fig. 2C and 2D). In Exp1, T3 leaf size differed significantly between HT and LT only for the last three leaves, whereas in Exp3, T3 total leaf area was similar between LT and HT groups.

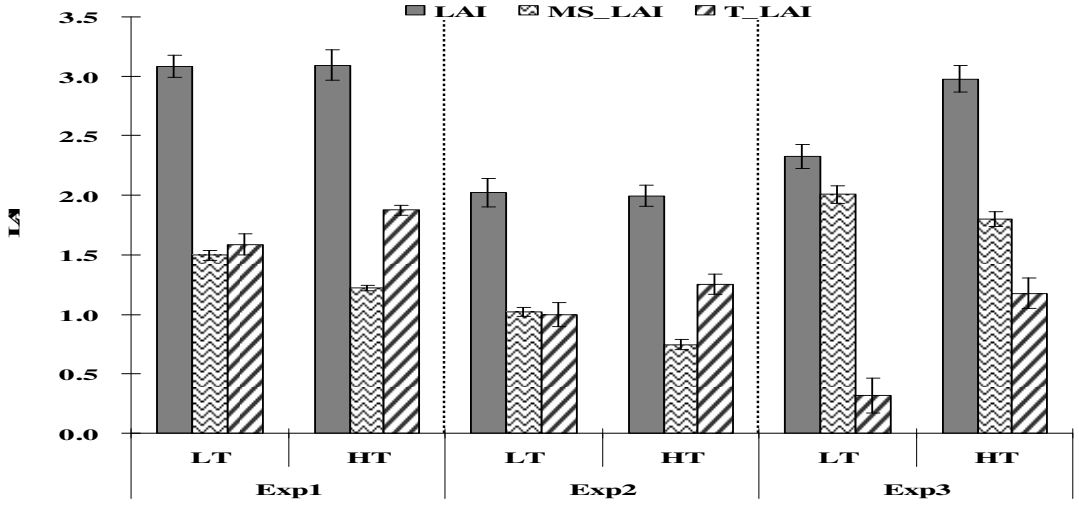

Figure 5: Total leaf area index (LAI) and its allocation between main stem (MS_LAI) and tillers (T_LAI) at flag leaf stage in the field environments. Hybrids with no significant difference were grouped into two groups: low tillering hybrids (LT: hybrids 1, 2 and 3) and high tillering hybrids (HT: hybrids 4 and 5). Error bars indicate s.e.m.

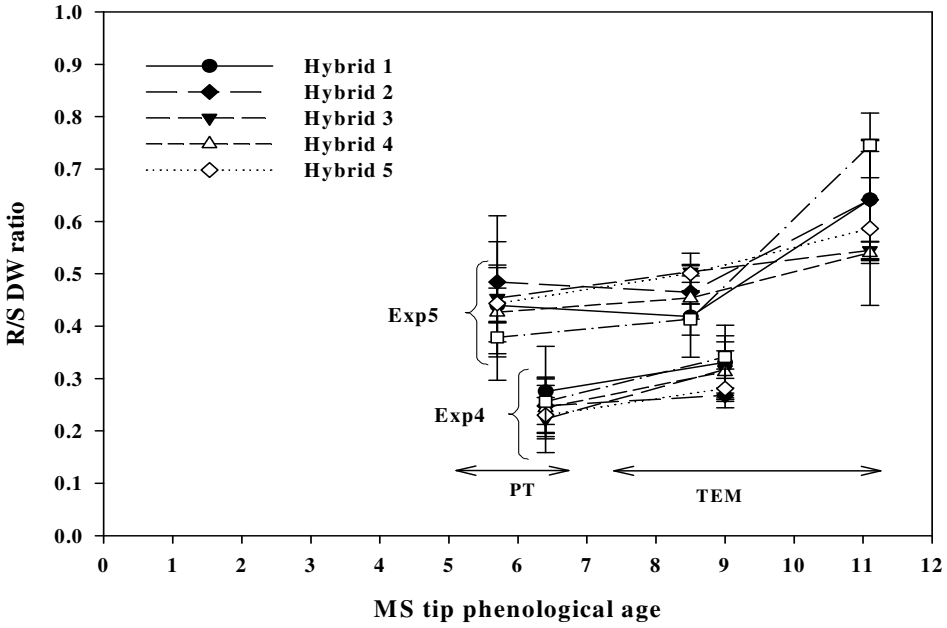

Figure 6: Dry matter partitioning between root and shoot (R/S DW ratio) prior to tiller emergence (PT) and during early tillering period (TEM) in controlled environments (Exp4 and Exp5).

Biomass accumulation

Results for biomass accumulation were consistent with results for LAI. Similarly to LAI, there were no genotypic differences in biomass accumulation within an experiment, either during the tillering phase or at flag leaf stage. The only exception was Exp3, where HT hybrids had a significantly higher biomass, associated with higher LAI. Consistent with these results, there were no genotypic differences in root mass (Exp4 and Exp5), root/shoot ratio (Fig. 6), blade-stem (including sheath) dry matter partitioning and SLA during tillering phase that could be correlated with high or low tillering behaviour. Plant SLA variation was largely explained by developmental stage and decreased from approximately 300$cm^2.g^{-1}$ (L5 stage) to 160 $cm^2.g^{-1}$ (flag leaf stage) in field experiments and from 500 $cm^2.g^{-1}$ (L4 stage) to 250$cm^2.g^{-1}$ (L8 stage) in controlled environment experiments while no measurable significant difference between high or low tillering hybrids within an experiment could be observed (data not shown).

As differences in tillering occurred in the absence of differences in biomass, the relationship between relative tillering rate (RTR) and relative growth rate (RGR) was different for HT and LT hybrids (Fig. 7). For both groups of hybrids, a linear relationship with similar intercept with the x-axis existed. However, for HT hybrids, the slope of the relationship (0.65±0.05) was significantly higher than for LT hybrids (0.45±0.04).

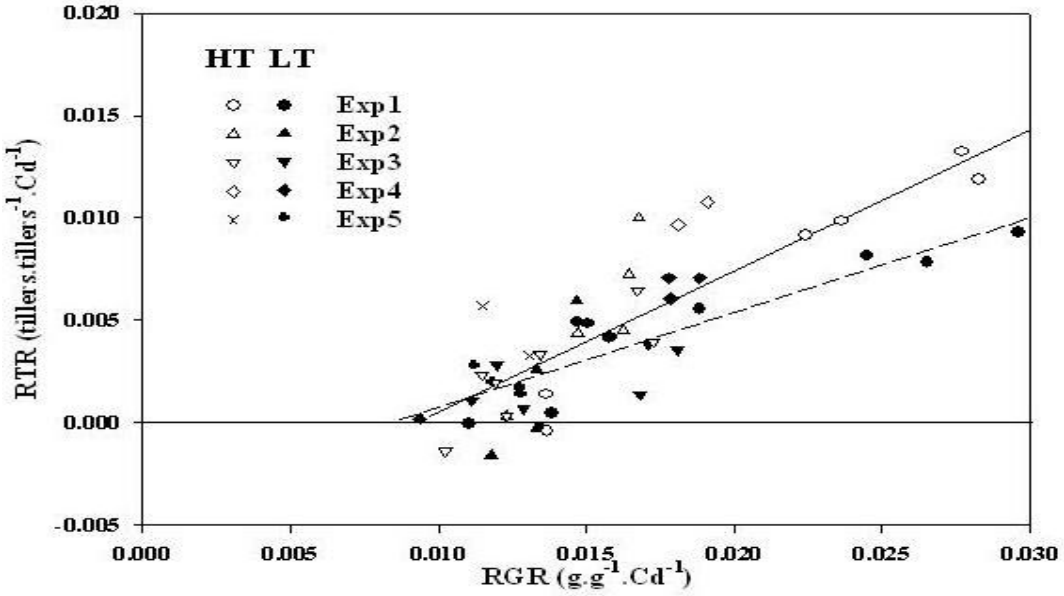

Figure 7: Relationship between RGR and RTR for high tillering (HT: empty symbols with solid line regression y=0.65x-0.005, R^2=0.74***) and low tillering (LT: filled symbols with dotted line regression y=0.45x-0.003, R^2=0.6***) group for each experiment from tiller emergence to cessation of new tillers.

Development of a S/D index integrating genotypic effects on tillering

The relationship between tillering and leaf width (Fig. 4) is consistent with the hypothesis that genotypic differences in tillering are associated with differences in the internal plant competition for assimilates. Therefore, a S/D_{index} was developed by incorporating the genotypic factors controlling tillering from the current paper into the S/D_{index} developed to consider environmental control of tillering (Eq. 1) developed by Kim *et al.* (2008). Because genotypic differences in tillering were associated with differences in leaf width, the S/D_{index} was altered by adding a term for the LWIR into Eq. 1. The S/D index was thus computed as:

$$S/D_{index} = \frac{RAD_{LED5} \times LA_{L5} \times DevPhase}{TT_{LED5} \times LLIR \times LWIR} \quad [2]$$

Tillering rate was computed as average tiller number per tip-phyllochron from the potential appearance time of T1 until the cessation of tiller appearance (i.e. [TEM]). The relationship between S/D_{index} (Eq.1) and tillering rate differed for HT and LT hybrids (Fig. 8) but much of this genotypic effect that was present in the relationship between TNmax and LWIR (Fig. 4), was removed in the relationship between tillering rate and this revised S/D_{index} (Eq.2 and Fig. 8). The slopes of the relationships were similar (0.025) for HT and LT hybrids, but the intercept with the x-axis was significantly lower for HT hybrids. However, within this HT group, hybrid 5 had a low tillering rate if the S/D_{index} was low, whereas tillering in hybrid 2 did not always respond linearly to the S/D status (Fig. 8).

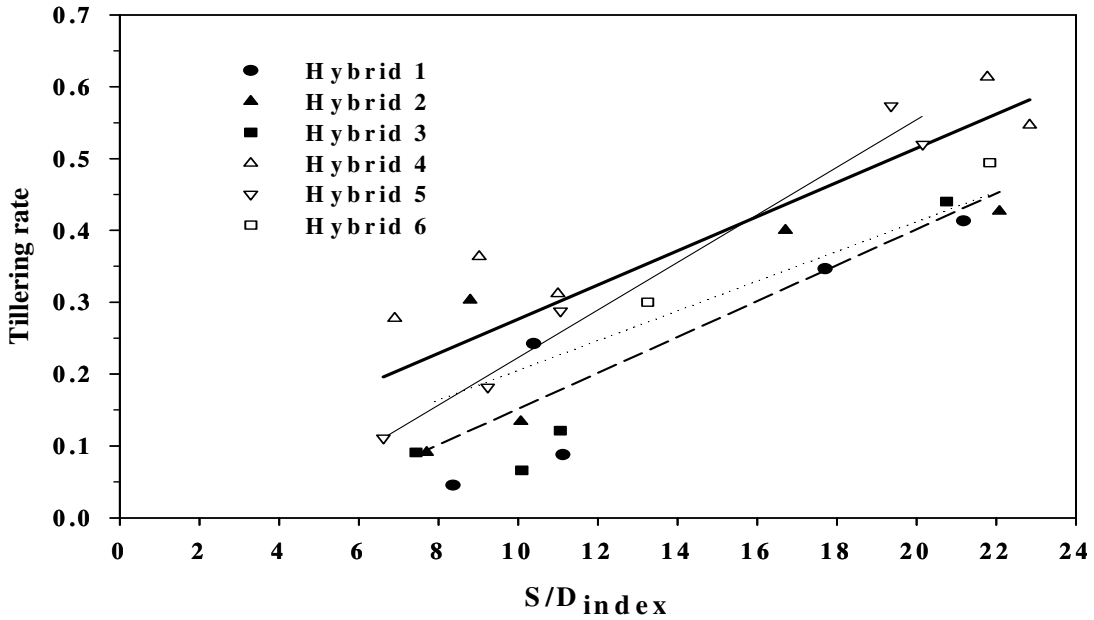

Figure 8: Relationship between leaf width increase between main stem L4 and L9 (LWIR) and maximum tiller number (TNmax) for each hybrid in each respective experiment. Solid lines represent linear regressions of all hybrids in each experiment.
(B) Tillering response to S/D index integrating genotypic difference in MS leaf size (LWIR) in the plant demand component. Solid line in bold represents linear regression for HT hybrids group (y=0.024x+0.039, R^2=0.84**) and dashed line in bold represents regression for LT hybrids group (y=0.025x–0.089, R^2=0.77**). Solid line in light (y=0.033x+0.1, R^2=0.97***) and dotted line (y=0.021x+0.001, R^2=0.7*) represents a separate linear regression for line 5 and line 2 respectively.

CHAPTER III

DISCUSSION

This study quantified genotypic differences in tillering of sorghum through a carbohydrate S/D_{index} established on five hybrids obtained from lines in a BC1F4 population that segregated for tillering, cropped across a range of high- and low-tillering environments. Results showed that genotypic differences in tillering were associated with differences in leaf width, which resulted in early differences in carbon availability for tillering at the whole plant. As a consequence, genotypic differences in tillering were predominantly due to differences in the frequency of appearance of tillers at lower leaf ranks. A generalised S/D_{index}, that incorporated both genotypic and environmental effects on tillering, explained a large proportion of the observed variation in tillering, although it did not capture all the genotypic effects. The analysis suggested that hybrids may also differ in the supply/demand threshold at which tillers appear.

Relationship between leaf size and tillering

High-tillering hybrids tended to have less main shoot leaf area than low-tillering hybrids (Fig. 5), and this difference was associated with smaller leaf size (Fig. 3), in particular narrower leaves (Table 4), rather than with differences in phyllochron or leaf number (Table 3). This is consistent with observations for pearl millet (van Oosterom *et al*., 2001b). An effect of leaf width, rather than leaf length, on tillering is consistent with results for wheat (Rebetzke *et al.*, 2004) and rice (Tivet *et al.*, 2001) showing that maximum leaf width is under more direct genetic control than leaf length, the latter being more prone to phenotypic plasticity (Kaitaniemi *et al.*, 1999; Lafarge and Tardieu, 2002). In *Arabidopsis*, two independent developmental processes of leaf morphogenesis was identified, i.e leaf length and leaf width development, with specific genes regulating meristematic activities in cell division and proliferation (Tsuge *et al.*, 1996; Tsukaya, 2005). Leaf width seems to be determined by genetic determinants in the lateral expansion and this could be linked to

meristem size. Similarly, Kim *et al.* (2008) showed for a single sorghum hybrid a higher variability across environments for leaf length than leaf width. In general, HT and LT hybrids had similar slopes for the relationship between S/D_{index} and tillering (Fig. 8), highlighting the important role of leaf size (leaf width) in capturing genotypic differences in tillering.

Regulation of tillering through a supply/demand framework

The genotypic differences in tillering were predominantly due to a difference in the frequency of appearance of tillers in the axils of lower rank leaves (Table 2). The processes determining genotypic differences in tiller appearance thus operated at the onset of tiller outgrowth. This supports the hypothesis that genotypic differences in the early development and growth of the main shoot are critical determinants of genotypic differences in tillering (Bos and Neuteboom, 1998b; Dingkuhn *et al.*, 2001). This points to control by either supply/demand or hormones.

The impact of S/D on tillering is supported by the observation that genotypic differences in tillering were associated with differences in main leaf area around tiller emergence. In the absence of genotypic differences in the appearance of tillers of a specific rank (Fig. 2), and of differences in leaf appearance rate (Table 3), the genotypic differences in leaf width (Table 4) and hence individual leaf size (Fig. 3) must have resulted in differences in main stem leaf area at the onset of tiller appearance. The importance of high main stem leaf area early in the season in restricting sorghum tillering has also been noted by van Oosterom *et al.* (2008), although in that study, differences in main stem leaf area were a consequence of differences in leaf appearance rate, rather than leaf size. Although we did not observe any consistent differences between HT and LT hybrids in main stem leaf dry weight at the onset of tillering in the biomass samples (data not shown), it is likely that was due to the inevitable lack of resolution in the biomass samples.

The root/shoot partitioning before first tiller emergence or during early tiller

outgrowth (controlled environment experiments) did not show any significant genotypic difference related with tillering ability (Fig. 6), which was consistent with recent results for wheat (Palta *et al.*, 2007). Moreover, there were no consistent genotypic differences in blade-stem partitioning and SLA. Therefore, it is likely the difference in leaf area generated a different carbohydrate S/D state for LT or HT hybrids.

The higher tillering of HT hybrids was also associated with a higher propensity to tiller at a given RGR rather than to a higher RGR (Fig. 7). This is in contrast to results for rice, where genotypic differences in RTR are associated with differences in RGR (Dingkuhn *et al.*, 2001). As a consequence, there was a significant difference in the value of the S/D_{index} at which tillering started between genotype groups (Fig. 8). These results suggest that there is a difference in the threshold S/D_{index} at which tiller buds start to grow. This difference could be due to hormonal signalling or responsiveness to sugar levels in the plant.

Our results support the hypothesis that genotypic differences in tillering were associated with differences in the carbon supply demand balance, associated with leaf width and in the threshold value at which tillers grew out, possibly associated with sugar levels or hormonal signalling. An estimation of internal competition state between high and low tillering ability is therefore required to integrate a genotype-dependent threshold parameter (intercept with x-axis) in a model framework to correctly quantify tillering response.

Implications for adaptation to drought

The genotypic differences in leaf size, and consequently in tillering, could result in differences in leaf area dynamics over time, which could ultimately affect leaf area at anthesis (Fig. 5) and hence adaptation to drought (Borrell *et al.*, 2000; van Oosterom *et al.*, 2008; Hammer, 2006). The larger main shoot leaf size (Fig. 3) of LT hybrids would indicate a higher LAI early in season. In environments without genotypic differences in LAI at anthesis (Fig. 5), this difference in main stem leaf size could result in higher LAI for most of pre-anthesis

period, resulting in more water use of LT hybrids. However, in environments where differences in tillering are substantial (e.g. Exp3, Fig. 5), a higher tiller LAI in HT hybrids can more than compensate for their lower main stem LAI, resulting in a smaller plant size for LT hybrids and hence (in the absence of differences in transpiration efficiency), in lower water use by LT hybrids. These interactions illustrate that the effect of tillering (leaf size) on drought adaptation is not straightforward and depends on specific environmental conditions and management practices.

To provide a better insight into complex genotype*management*environment (G*M*E) interactions, the understanding of G and E effects on the dynamics of tillering generated in this study could be incorporated into suitably structured crop growth simulation models (Keating *et al.*, 2003; Luquet *et al.*, 2006). Provided the input parameters of the tillering model are closely associated with QTLs, the model could then be used as a tool to scale information at the QTL level up to consequences at the crop level (e.g. grain yield), including G*M*E interactions. The validity if this approach has already been demonstrated by Chenu *et al.* (2008), who incorporated a QTL model for leaf elongation rate of maize into a crop-level simulation model. For tillering, incorporation of the framework into a crop growth simulation model could provide better insights into the effects on grain yield of the interactions between genotypic differences in tillering and management practices, such as the skip row system used in water limited environments (McLean *et al.*, 2003). This could potentially greatly improve the efficiency of marker-assisted selection for drought adaptation.

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"As for man, his days are like grass,
he flourishes like a flower of the field;
the wind blows over it
and it is gone,
and its place remembers it no more."

"At least there is hope for a tree:
If it is cut down, it will sprout again,
and its new shoots will not fail.
Its roots may grow old in the ground
and its stump die in the soil,
yet at the scent of water it will bud
and put forth shoots like a plant."

CHAPTER IV

A genetic analysis of tillering in sorghum using model-based phenotyping

Kim Hae Koo[1,2]

Luquet Delphine[1]

van Oosterom Erik[2]

Dingkuhn Michael[1]

David Jordan[3]

Hammer Graeme[2]

[1] CIRAD, UPR 59, Avenue Agropolis Lavalette TA A 59/01 34398 Montpellier, France

[2] The University of Queensland, School of Land, Crop and Food Sciences, Brisbane QLD 4072, Australia

[3] Queensland Department of Primary Industries & Fisheries, Hermitage Research Station, Warwick, QLD 4370, Australia

ABSTRACT

• *Background and Aims* A better understanding of the effects and function of QTLs that control tillering in sorghum can improve the efficiency of selection for this trait, which is important for drought adaptation. The aim of this paper is to identify QTLs associated with tillering by phenotyping for attributes derived from a modelling framework for tillering in sorghum.

• *Methods* Eight BC2F2 mapping populations of sorghum, each consisting of 50-100 plants, were grown in the field at Gatton, SE Queensland. Traits selected for phenotyping were based on a dynamic modelling framework that was developed previously. These included the size of individual leaves that appeared during tillering, the presence of tillers at specific leaf ranks, and a calculation of the threshold carbon supply/demand index at which tillers grow out. Leaf tissue samples were collected from each individual plant and genotyping was performed using SSR markers.

• *Key Results* We identified three QTLs associated with tillering, The QTLs on LG3 and LG4 affected tillering through and effect on the threshold and hence the appearance of lower rank tillers. In addition, the QTL on LG4 affected leaf size through leaf width. A QTL on LG9, by contrast, was not associated with either leaf size or the threshold. Based on these QTL functions, the QTLs were incorporated into a dynamic framework for the genetic control of tillering.

• *Conclusions* This study identifies genomic regions associated with tillering in sorghum and illustrates how a modelling framework can assist genetic studies on complex, adaptive traits in a manner relevant to breeding programs.

Key words:

Tillering, complex trait dissection, modelling, plant physiology, genetics, QTL

INTRODUCTION

Tillering in sorghum is a complex plant trait of interest as it is a key component of canopy development, which ultimately has significant impact on grain yield, either positive or negative depending on seasonal conditions and crop management (Hammer et al., 1996). Tillering in sorghum is known to be regulated by genetic and environment effects (Lafarge ref; 2 Kim et al. papers). In those previous studies on tillering we developed a modelling framework that explained the dynamics of tillering as a consequence of plant internal competition for assimilates (C). We used the concept of the ratio between C supply and demand (S/D), and developed a measure of S/D to index the level of plant internal competition. Supply was related to measures of light interception and assimilation while demand was related to rate of organ growth. Aspects of both S and D were found to be dependent on genotype and/or environment attributes.

Recently it has been suggested that crop physiological understanding integrated into a robust crop modelling framework provides an avenue to advance the genetic analysis of complex traits and thus enhance opportunities for effective molecular breeding (Yin *et al.*, 2004; Hammer *et al.*, 2006). An effective modelling framework can provide a dynamic dissection of the component traits and/or processes underpinning the complex trait of interest. Hence, the parameters of such a dynamic modelling framework provide a means to link to underpinning genomic regions in a way that might reduce the environment and genetic background context dependencies that impede molecular breeding using conventional statistical QTL models (Cooper *et al.,* 2005; Hammer *et al*., 2005; Baenziger *et al.*, 2004). By improving gene-to-phenotype predictive capabilities, the consequences of combining various genomic regions can be foreshadowed more reliably for any specific production environment. Chenu et al. (2008) present an example of this approach in maize by developing a model capable of linking the stable QTL associated with leaf expansion rate (Reymond, 2003) to phenotypic consequences on crop growth and yield.

In this study we aim to identify QTLs associated with tillering in sorghum by phenotyping relevant populations for attributes derived from the modelling framework developed earlier. Hence, in addition to identifying genomic regions associated with tillering in sorghum, the study provides a test of the concept of using model-based approaches to genetic analysis of complex traits

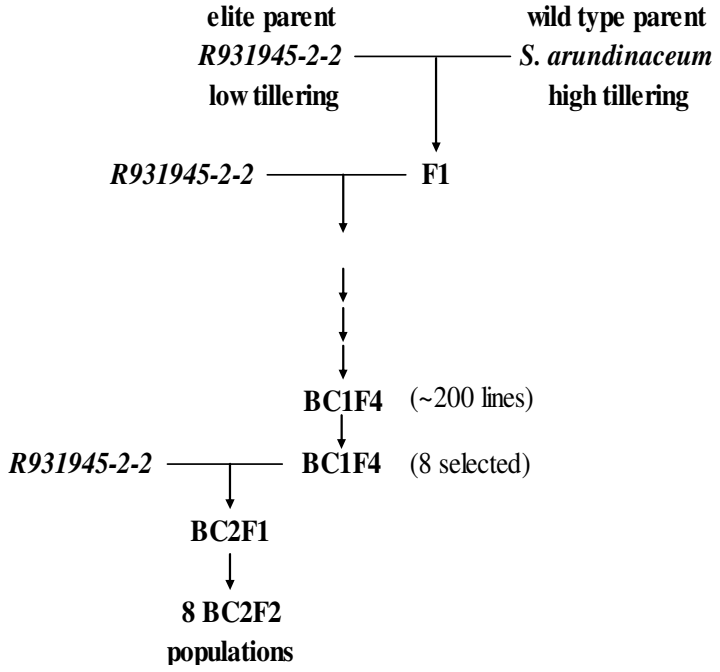


Figure 1: Pedigree of the eight BC2F2 mapping populations generated from an initial cross between an elite line, 31945-2-2, and *Sorghum arundinaceum*.

BC2F2 Population name	Initial cross (elite parent x BC1F4 selected line ID)	BC1F4 line Tillering potential	Number of BC2F2 lines phenotyped
F2_R05422	ms3*5_R931945-2-2 x **R999017**	High	92
F2_R05429	ms3*5_R931945-2-2 x **R999100**	High	89
F2_R05434	ms3*5_R931945-2-2 x **R999197**	High	92
F2_R05430	ms3*5_R931945-2-2 x **R999110**	High	52
F2_R05426	ms3*5_R931945-2-2 x **R999081**	High	29
F2_R05425	ms3*5_R931945-2-2 x **R999066**	Low	88
F2_R05436	ms3*5_R931945-2-2 x **R999218**	Low	92
F2_R05421	ms3*5_R931945-2-2 x **R999003**	Low	40

Table 1: Summary of BC2F2 population name, initial cross (BC1F4 parental line ID), tillering potential and number of BC2F2 lines phenotyped.

MATERIALS AND METHODS

Genetic material (mapping populations)

The experiment included eight BC2F2 populations, derived from eight BC1F4 inbred lines (Fig.1). The lines were part of an advanced backcross (31945-2-2//31945-2-2/*S. arundinaceum*) population of over 200 inbred lines. 31945-2-2 is a low-tillering elite tester developed by the Q-DPI&F (Queensland Department of Primary Industries & Fisheries) breeding program and *Sorghum arundinaceum* (*S. bicolor* ssp. *arundinaceum*) is an African wild type sorghum known for its high tillering ability. The eight lines were selected for contrasting tillering behaviour (Tab.1), and comprised three low tillering (LT) lines (R999003, R999066, and R999218) and five high tillering lines (R999017, R999081, R999100, R999110, and R999197). Five of these lines (R999017, R99066, R99100, R999197, and R999218) had been selected for similar anthesis date and plant height (Kim *et al.*, 2008b).

Experimental set up

Each of the eight BC2F2 populations was sown in a single row in a field experiment at Gatton, Australia (27°34'S, 152°18'E, 94 m asl) on 11 Jan. 2007. Row spacing was 1 metre and the experimental field size was 12 rows of 36 metres including 2 border rows on either side. To ensure a homogeneous density of 5 plants per linear meter, 4 seeds were hand-planted every 20cm and thinned to a single plant around the 3rd fully expanded leaf, resulting in a density of 5 plants m^{-2} A basal fertiliser was applied prior to sowing and fertiliser and supplemental irrigation were managed to ensure optimum growing conditions until maturity. Atrazine was applied after sowing, prior to emergence, to control weeds. Insecticides and fungicides were applied as necessary to control heliothis and rust. Air temperature (T, minimum, maximum and average with Campbell Scientific 108-L6), relative humidity and global solar radiation (Rad in MJ.m^{-2}.day^{-1} with Li-Cor Li200S) were measured at 1.5m above

the soil surface. Data were recorded hourly using a datalogger (CR10, Campbell Scientific). The experiment was conducted until physiological maturity.

Phenotyping of key tillering components

Phenotyping was conducted on 50-100 plants in each population. The traits selected for phenotyping were based on a carbon supply/demand index that integrates genotypic and environmental effects on tillering (Kim *et al.*, 2008a,b). They included observations on tillering, leaf number, phenology, plant height, plus some derived parameters.

Leaf size phenotyping consisted of measuring the length and maximum width of the blades of main stem Leaf 5 to Leaf 9 on all plants. Individual leaf area was computed by multiplying length and width by a shape coefficient of 0.695 (Hammer *et al.*, 1993; Lafarge *et al.*, 2002). The leaf length increase rate (LLIR) and leaf width increase rate (LWIR) were determined for each plant as the slope of the linear regression of the length or width of successive leaves on leaf position, between main stem leaf 5 and leaf 9 (Kim *et al.*, 2008a,b).

Tillering was phenotyped for each plant by the presence or absence of each tiller rank (T1 to T6), the maximum tiller number produced (TNmax) and the fertile tiller number at anthesis (FTN). Tillers were labelled according to the axil of the main shoot leaf from which they emerged, with T1 emerging from the axil of main shoot Leaf 1. As previous experiments (Kim et al., 2008b) showed that genotypic differences in tillering were associated with the LWIR and with a S/D threshold at which tillers appear, the linear regression between LWIR and TNmax was used to determine a tillering threshold (thr) for each plant within a population. The threshold represented the difference in observed TNmax and the expected TNmax for a plant, based on the LWIR and the regression between TNmax and LWIR for the particular population (Fig 2.).

Phenological parameters observed included the total number of leaves on the main stem and number of days from sowing to anthesis. The date of anthesis was determined for

each plant when over 50% of anthers had exerted on the main stem panicle. Plant height was measured as height from ground level to the ligule of the flag leaf on the main stem.

Selective genotyping strategy

The eight BC1F4 parental lines were genotyped with DArT technology (Jaccoud et al., 2001), using the protocol detailed by Mace *et al.* (2008). This genotyping was part of a diversity analysis in sorghum (Mace *et al.*, 2008) and allowed the identification of genomic regions that were still segregating, an indication of introgression of S. *arundinaceum* in the recurrent parent background (R931945-2-2). Segregating regions among the BC1F4 lines were screened with a sorghum consensus map that included SSR markers. We used this consensus map to select SSR markers (Table 2) around the segregating regions identified by DArT markers and these selected SSR markers were used for genotyping of selected BC2F2 individual plants. QTL analysis was first performed with single-marker regression, to test independence in segregation between one SSR and field observations. A similar approach was used to detect putative QTLs among the segregating regions linked with the plant parameters identified within our modelling framework.

Table 2: Localisation and distance on linkage group (LG) of SSR markers (according to a consensus map (Mace *et al.*, unpublished) screened for polymorphism on BC1F4 lines. Expected PCR amplification product size, repeat motif and annealing temperature (Tm) are indicated.

SSR marker	LG	Distance (cM)	Expected Size	Repeat Motif	Tm
Xtxp357	1	62.5	~273	$(GT)_{10}$	55
Xtxp43	1	65.8	~171	$(CT)_{28}$	60
Xtxp88	1	67	~144	$(AG)_{31}$	53
Xtxp149	1	71	~169	$(CT)_{10}$	55
Xtxp32	1	77	~133	$(AG)_{16}$	60
Xtxp37	1	92	~189	$(TC)_{23}$	55
Xtxp335	1	98.2			55
Xgap57	1	109.4			60
Xtxp58	1	109.7	~160	$(AG)_{13}$+$(GA)_{16}$	
Xtxp75	1	115.5	~172	$(TG)_{10}$	50
Xtxp279	1	115.5			55
Xtxp25	2	20	~139	$(CT)_{12}$	55
Xtxp500	3	47.2			61
Xtxp461	3	56.3			62
Xtxp33	3	59.8-62.8	~221	$(TC)_{20}C(TG)_5$+$(CT)_9CC(TG)_7$	55
Xsb5-236	3	62.8	165-185		
Xtxp205	3	65.1-71.1	~211	$(AG)_{12}$	55
Xtxp31	3	71	~222		60
Xtxp336	3	73.2			55
Xtxp183	3	75.2	~190	$(TG)_9$	55
Xtxp444	3	80.2			60
Xtxp120	3	87.5-89.2	~217	$(AT)_{18}$	55
Xtxp506	4	0			61
Xtxp504	4	6.1			62
Xtxp343	4	73	~155	$(AGT)_{21}$	55
Xtxp12	4	71.4-75.8	~193	$(CT)_{22}$	55
Xtxp41	4	107	~278	$(CT)_{19}$	55
Xtxp21	4	153	~179	$(AG)_{18}$	60
XSb5-214	4	97.1 ?		?	?
Xtxp95	6	125.7			53
Xtxp176	6	134.1-135.5	~161	$(AG)_4AAC(GA)_4$	55
Xtxp57	6	141			55
Xtxp17	6	146	~164	$(TC)_{16+}(AG)_{12}$	55
Xgap342	7	71.9			
Xtxp278	7	74.1			
Xtxp92	7	87.8	~170	$(GAA)_5$	50
Xtxp295	7	124.5	~165	$(TC)_{19}$	55
Xtxp339	9	35.9-43	~202	$(GGA)_7$	55
Xtxp10	9	67.2	~145	$(CT)_{14}$	50
Xtxp67	9	87.9	~175	$(GA)_{28}$	55
Xtxp230	9	90.1	~191	$(GA)_{28}$	55
Xtxp410	9	115.4			59
Xtxp459	9	120			*54*
Xtxp358	9	123.1-128.9	~267	$(ATT)_{22}$	55
Xtxp289	9	132.3	~249	$(CTT)_{11}CTC(CTT)_{16}$	55

DNA collection, extraction, PCR and SSR markers optimisation

Mature leaf tissues of approximately 50-90 plants per populations (Fig. 1) were collected in 96-well plates around the flag leaf stage in the field. Genomic DNA of each genotype was extracted by the Mixer Mill 300 high throughput system according to the protocol of Tanksley à la Paul, modified by J. Carling. For each SSR that was screened, polymerase chain reaction (PCR) conditions were optimised by adapting the annealing temperature (Tm), the concentrations of $MgCl_2$, Taq, buffer, water and dNTPs, and the amount of DNA of BC1F4 or BC2F2 lines (quality checked using agarose gel electrophoresis). PCR-generated DNA products were visiualised by polyacrylamide gel electrophoresis using GelScan 3000 (Corbett Research) and a laser for DNA detection. One microlitre of each PCR product was loaded onto a 4% non-denaturing TBE-polyacrylamide gel and pulse-loaded for 10 s. Approximately 15 mL solution was used for each gel [1.5 mL 40% acrylamide (37:1 bisacrylamide : acrylamide) (Sigma), 12.225 mL ddH_2O, 0.9 mL 10 × TBE, 0.375 mL 80% glycerol]. 30 µL of TEMED and 75 µL 10% ammonium persulphate were added to start polymerization prior to pouring the gel. Gels were run for approximately 40 min (1200 V, 37°C) with 0.6 x TBE buffer.

Based on the consensus map, over 50 SSRs around the polymorphic regions according to the DArT genotyping data of the eight BC1F4 parental lines (less than 10cM either side of the DArT maker) were identified and screened for polymorphism on the same BC1F4 (Table 2). Eventually, eight polymorphic SSR markers, located on four different linkage groups (LG), were chosen to genotype selected plants (based on contrasting phenotype) of selected BC2F2 populations: Xtxp88 and Xtxp149 on LG1; Xtxp500 and Xtxp31 on LG3; Xtxp343 and Xtxp12 on LG4; Xtxp67 and Xtxp410 on LG9 (Table 2).

Data analysis using R/qtl package in R

Statistical analyses were performed in R (R Development Core Team, 2007) and QTL analysis using R/qtl package (Broman *et al.*, 2003). The "*scanone*" function was used to execute a single QTL model (QTL analysis according to Lander and Botstein (1989) method). Either a normal or binary model was used according to the phenotype trait distribution (e.g. binary model for traits such as specific tiller rank presence or absence). Each BC2F2 population was taken as a covariate and individuals with missing genotypes were discarded. The presence of QTL was determined according to a single marker regression analysis by identifying individual SSR markers with significant association with phenotypic trait variation, i.e. a LOD threshold value of >3.0 (Churchill and Doerge, 1994).

RESULTS

BC2F2 population phenotyping

Table 3: Summary of the phenotypic trait means and standard deviation (StDev) of lines in each BC2F2 populations

F2_#	F2_R05422		F2_R05429		F2_R05434		F2_R05430		F2_R05426		F2_R05425		F2_R05436		F2_R05421	
	Avg	StDev	Avg	StDev	Avg	StDev	Avg	StDev	Avg	StDev	Avg	StDev	Avg	StDev	Avg	StDev
L5 Length	20.5	1.4	18.3	2.4	19.7	2.2	18.9	1.1	19.7	1.8	20.4	1.7	19.6	1.5	21.7	1.3
L6 Length	27.1	1.7	24.6	2.3	25.5	1.6	24.8	1.8	26.6	2.0	26.8	2.0	25.4	1.9	27.3	4.4
L7 Length	32.6	1.9	32.3	2.5	32.0	1.9	31.7	2.0	32.7	2.0	32.4	2.1	32.7	1.8	34.4	1.7
L8 Length	38.4	2.2	38.5	2.7	37.9	2.1	37.7	2.4	38.4	2.5	38.9	2.0	38.4	1.8	39.8	2.5
L9 Length	44.9	2.3	44.6	2.7	45.0	2.5	44.8	2.2	45.8	2.8	46.7	2.5	44.7	2.0	47.4	2.4
L5 Width	2.7	0.3	2.4	0.3	2.6	0.2	2.6	0.2	2.6	0.3	2.5	0.2	2.6	0.3	3.0	0.2
L6 Width	3.6	0.3	3.2	0.3	3.5	0.3	3.5	0.3	3.5	0.3	3.7	0.3	3.6	0.4	4.0	0.3
L7 Width	4.7	0.3	4.3	0.4	4.5	0.3	4.5	0.3	4.5	0.3	4.7	0.3	4.6	0.3	5.1	0.3
L8 Width	6.1	0.4	5.4	0.4	5.9	0.4	5.6	0.4	5.9	0.4	6.0	0.3	6.0	0.3	6.5	0.4
L9 Width	7.3	0.5	6.7	0.5	7.2	0.4	6.9	0.4	7.3	0.5	7.3	0.4	7.4	0.4	7.9	0.4
TN max	2.47	1.1	3.15	1.02	2.21	0.70	3.33	0.88	2.07	0.92	2.01	1.2	1.47	0.91	1.30	1.24
FTN	1.45	0.8	2.15	0.94	1.74	0.66	2.29	0.80	1.55	0.95	1.04	0.9	0.91	0.77	0.80	0.82
TN_T1	0.02		0.25		0.09		0.44		0.10		0.05		0.02		0.08	
TN_T2	0.61		0.93		0.85		1.00		0.83		0.42		0.43		0.38	
TN_T3	0.93		0.96		0.88		0.96		0.79		0.80		0.65		0.50	
TN_T4	0.68		0.72		0.38		0.63		0.31		0.48		0.29		0.30	
TN_T5	0.22		0.29		0.01		0.21		0.03		0.27		0.07		0.05	
TN_T6			0.06				0.08									
FTN_T1	0.00		0.11		0.03		0.85		0.03		0.00		0.00		0.03	
FTN_T2	0.35		0.84		0.78		0.92		0.69		0.23		0.29		0.23	
FTN_T3	0.78		0.82		0.78		0.38		0.62		0.58		0.46		0.35	
FTN_T4	0.32		0.35		0.14		0.02		0.21		0.14		0.15		0.18	
FTN_T5	0.00		0.02		0.00		0.00		0.00		0.08		0.01		0.03	
FTN_T6			0.00													
MS_LN	17.50	0.4	17.6	0.6	16.52	0.4	17.5	0.6	17.81	0.7	17.27	0.5	18.02	0.3	17.40	0.5
Anthesis	59 DAS		58 DAS		54 DAS		60 DAS		61 DAS		59 DAS		58 DAS		58 DAS	

Main stem phenology and plant height

The average leaf number per population varied from 16.5 to 18.0 leaves and there was approximately a week difference in the average anthesis date of the earliest and the latest population (55-62 days after sowing) (Table 3). Overall, only plants of the F2_R05434 population showed a significantly lower leaf number (16.5). As this population also reached anthesis a few days earlier than other populations, its leaf appearance rate was in general very similar to that of most other populations. Only the F2_R05430 and F2_R05421 populations, which combined late anthesis with average leaf number, were estimated to have a slightly slower leaf appearance rate.

For plant height (from ground level to flag leaf), all populations had a similar

distribution (average around 65cm). The only exception was the F2_R05430 population, which had slightly taller plants (average of 77cm).

Tiller number distribution in respective BC2F2 populations

The distribution of TNmax and FTN within BC2F2 populations (Fig. 2) was consistent with the tillering pattern of the BC1F4 parent (Fig. 1). High-tillering (HT) populations, compared to low-tillering (LT) ones, consistently had a lower fraction of plants that did not produce any tillers and tended to have a higher proportion of plants that produced 4 or more tillers. As a consequence, the peak in FTN frequency distribution was at 0-1 tillers for the LT populations, but at 2-3 tillers for HT populations (Fig. 2).

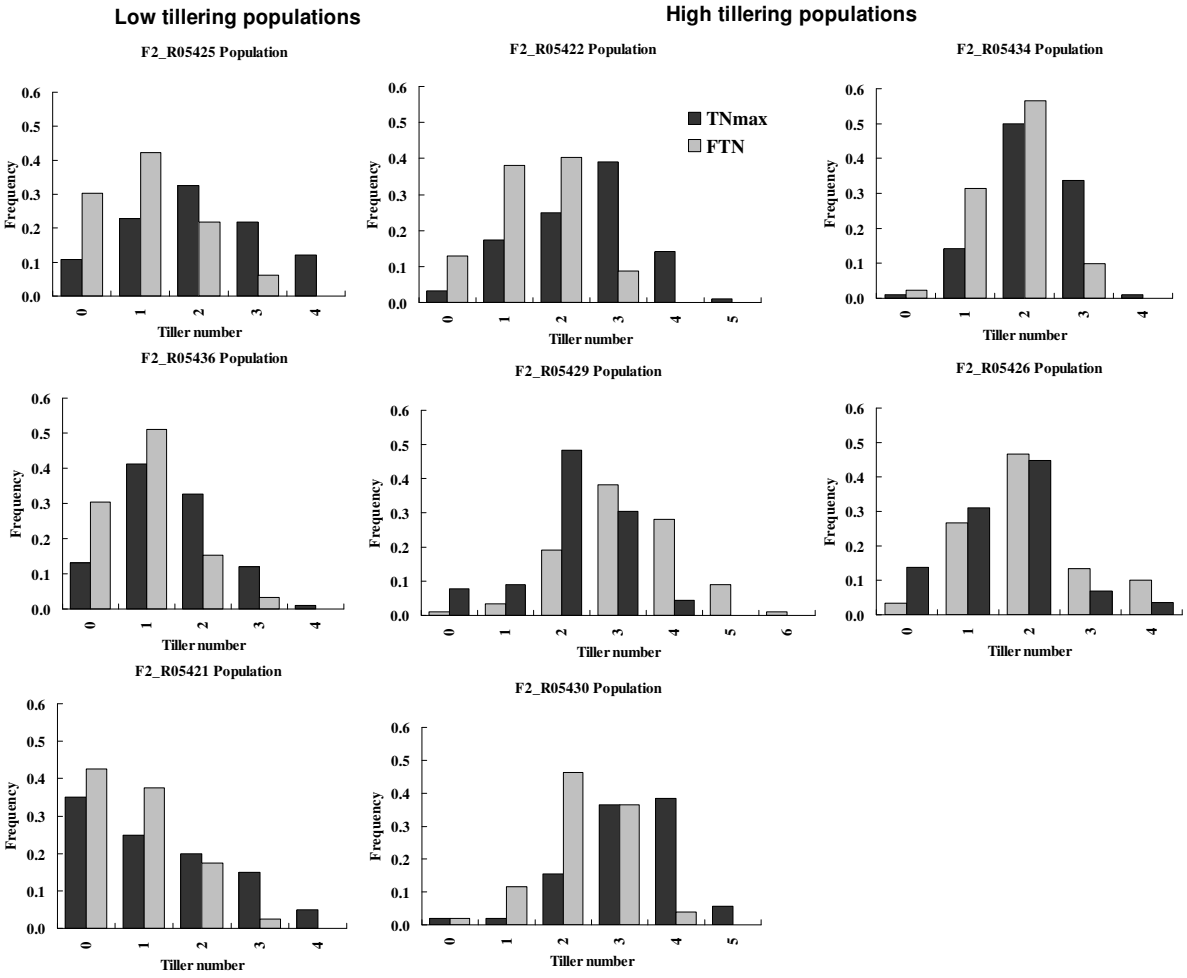


Figure 2: Distribution of tiller number (TNmax and FTN) within each population

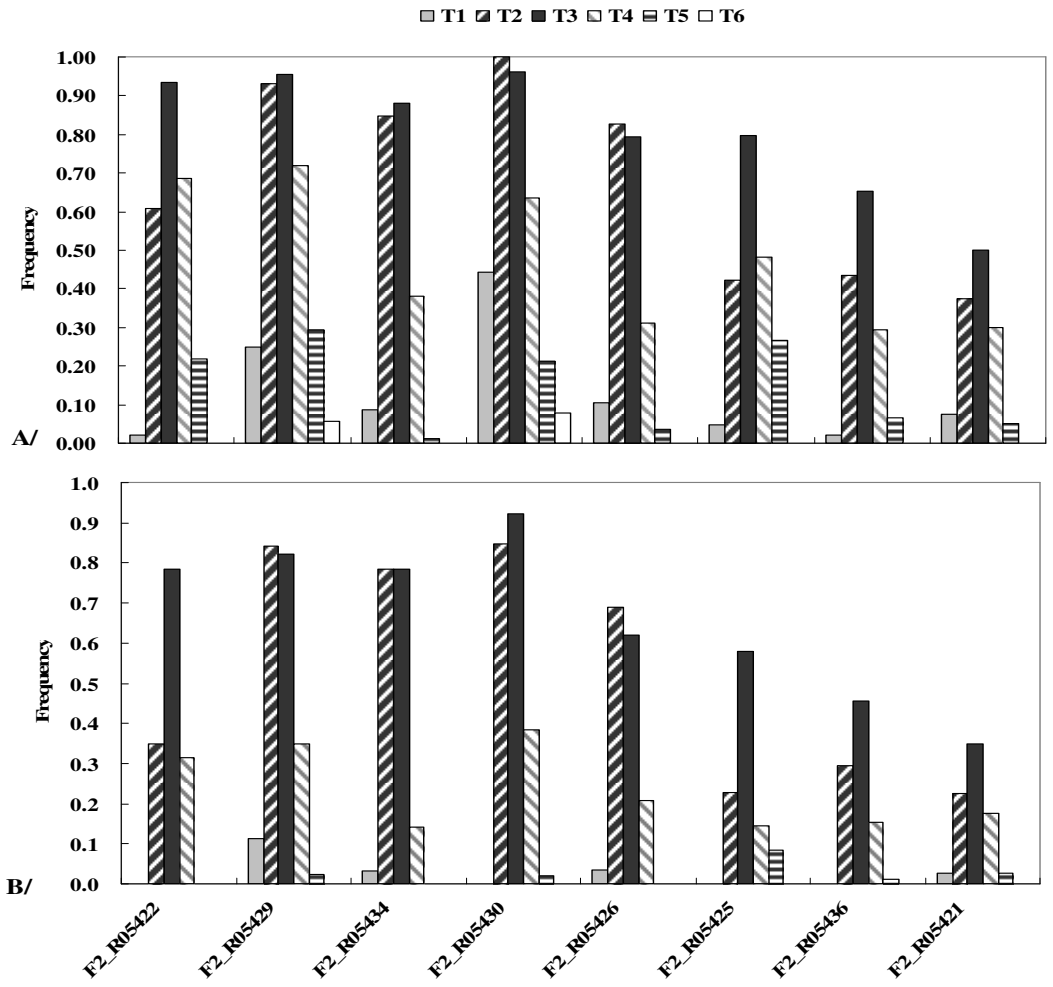
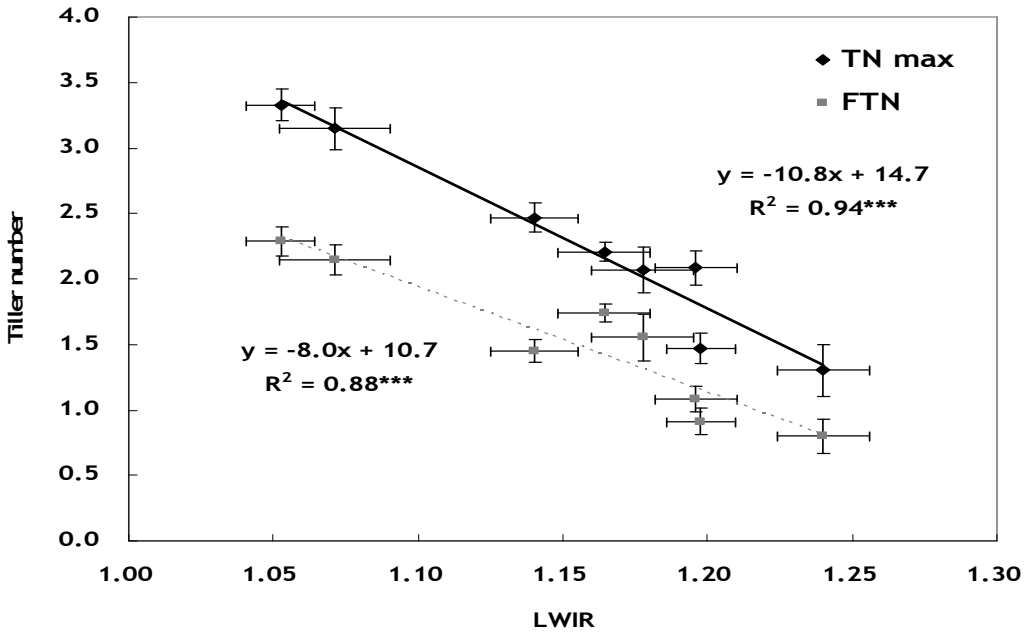


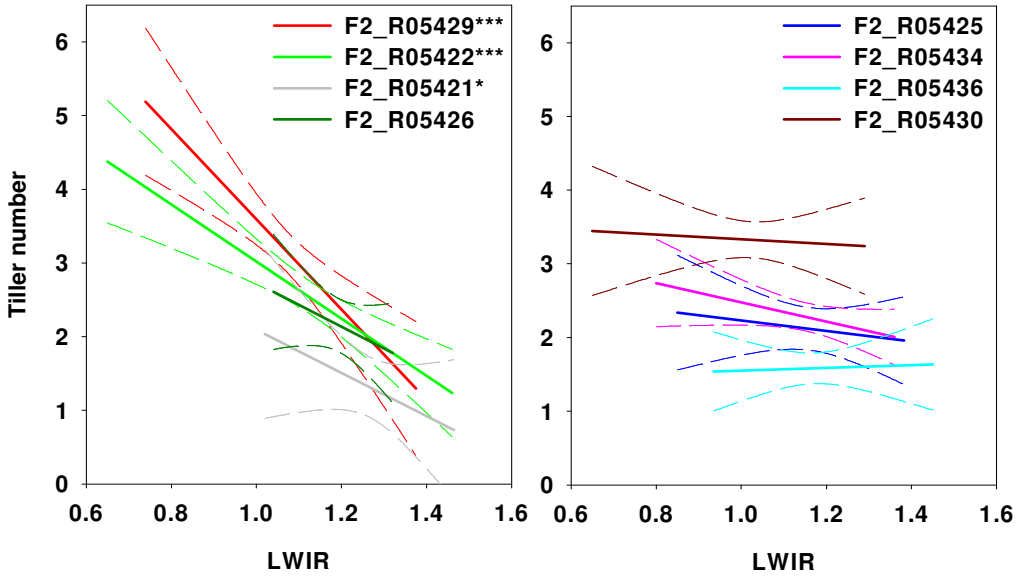
Figure 3: Distribution of each tiller rank (T1 to T6) appearance (A) and fertility (B) proportion among all lines for each BC2F2 population.

Tiller appearance and fertility frequency of each population

Tiller appearance and fertility frequency of each tiller rank (T1 to T6) for each population are presented in Fig. 3. The main difference between HT and LT populations was in the cumulated frequency of appearance of early tiller ranks (T1 and T2), which was either above or below 0.5 respectively. T3 was generally the tiller rank with the highest appearance and fertility frequency in LT populations, whereas in HT populations (except F2_R05422) T2 and T3 where the most dominant tiller ranks. The tiller survival rate was slightly higher for HT (70%) than for LT (59%) populations.



(A)



(B)

Figure 4: (A) Relation between average tiller number and LWIR of each BC2F2 population (LWIR vs. TNmax in plain line and LWIR vs. FTN in dotted line). s.e.m are represented for each population

(B) Relationship between leaf width increase between main stem leaf 5 and 9 (LWIR) and maximum tiller number (TNmax) among lines of a given population. Regression lines (plain lines) and 95% confidence interval (dashed lines) are represented for each population. (***) Significant regression at $p<0.001$; (*) at $p<0.05$

Relationship between leaf width and TNmax

Across populations, the average tiller number (either TNmax or FTN) was strongly negatively related to LWIR (Fig. 4A). Within populations, however, the tillering response to LWIR was not uniform. Three populations (F2_R05429, 22, and 26) showed a significant negative relationship ($p<0.05$) between LWIR and TNmax whereas the remaining populations (F2_R05425, 34, 36 and 30) did not show any significant relationship (Fig. 4B). In stead, they differed in their tiller number, irrespective of LWIR, suggesting a difference in the threshold at which tillers appear.

BC2F2 population genotyping

Selective Genotyping of the BC2F2 populations with SSR markers

DArT data of the BC1F4 parents identified four regions of interest around polymorphic DArT markers in LG1, LG3, LG4 and LG9F. Fig. 5 illustrates for each chromosome the regions with S. arundinaceum introgressions into the 31945-2-2 recurrent parent background, using GGT2 graphical genotype software (van Berloo, 2008).

Genotyping of the BC1F4 parental lines with all the SSR markers (Table 4) showed a number of interesting polymorphisms. On LG1, Xtxp88 and Xtxp149 on LG1 showed introgression from *S. arundinaceum* in four of the eight BC1F4 parents. Unfortunately, the optimisation protocol or DNA quality of the BC2F2 populations were insufficient for easy discrimination and scoring of PCR products. (Xtxp500 could not be scored clearly for BC2F2 progenies). Finally, the results presented here focus on four polymorphic SSRs (on 3 different chromosomes) which could be used across BC2F2 populations for consistency in amplification products by PCR and visualisation through an acrylamide gel electrophoresis (using Gelscan): Xtxp31 on LG3, Xtxp12 on LG4, Xtxp67 and Xtxp410 on LG9.

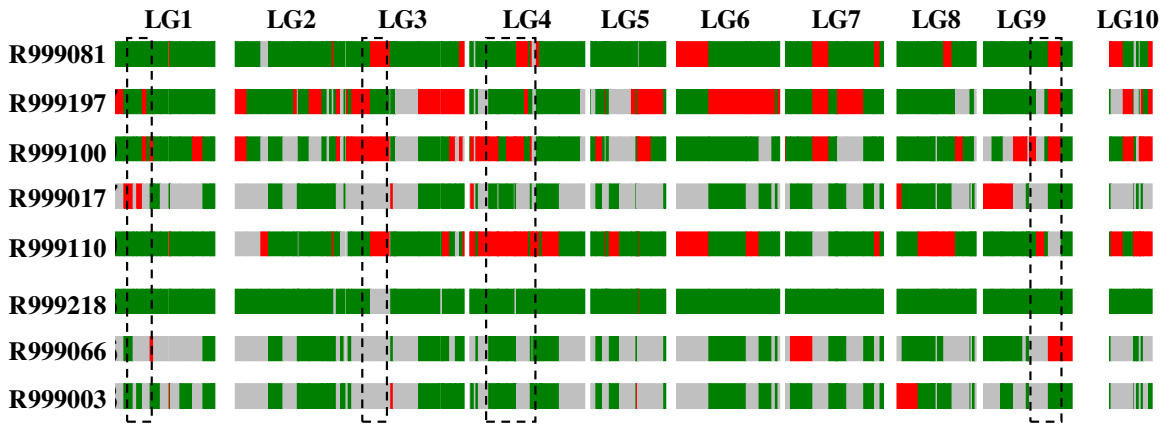


Figure 5: Visualisation for each chromosome (LG) *s.a.* introgressions (red) among 31945-2-2 (green) genetic background according to DArT genotyping data of BC1F4 lines (regions in grey represent missing data). From top to bottom for each LG: R999081, R999197, R999100, R999017, R999110 [high tillering lines], R999218, R999066 and R999003 [low tillering lines] respectively. In dotted frames are the regions targeted with selected SSRs (according to a consensus map) on LG1, LG3, LG4 and LG9.

Table 4: Polymorphic SSRs identified on BC1F4 parental lines. "A" represents homozygote marker similar to *s.a*; "B" represents homozygote markers similar to R31945-2-2; and "H" represents heterozygote locus.

SSR marker	LG	Distance	*sorghum arundinaceum*	31945-2-2 Low Tillering	R999017 High Tillering	R999100 High Tillering	R999197 High Tillering	R999110 High Tillering	R999081 High Tillering	R999066 Low Tillering	R999218 Low Tillering	R999003 Low Tillering
Xtxp88	**1**	**67**	**A**	**B**	**A**	**B**	**B**	**B**	**B**	**A**	**B**	**B**
Xtxp149		**71**	**A**	**B**	**A**	**A**	**B**	**A**	**B**	**B**	**B**	**B**
Xtxp500	**3**	**47.2**	**A**	**B**	**A**	**A**	**B**	**A**	**B**	**B**	**B**	**B**
Xtxp205		68	**A**	**B**	**A**	**A**	**B**	**A**	**B**	**B**	**B**	**B**
Xtxp31		**71**	**A**	**B**	**A**	**A**	**B**	**A**	**B**	**B**	**B**	**B**
Xtxp444		80.2	**A**	**B**	**B**	**B**	**B**	**A**	**B**	**B**	**B**	**B**
Xtxp504	**4**	6.1	**A**	**B**	**B**	**B**	**B**	**A**	**B**	**B**	**B**	**B**
Xtxp343		**73**	**A**	**B**	**H**	**A**	**B**	**A**	**A**	**B**	**B**	**B**
Xtxp12		**73.6**	**A**	**B**	**H**	**A**	**B**	**A**	**A**	**B**	**B**	**B**
Xtxp21		153	**A**	**B**	**B**	**B**	**B**	**A**	**B**	**B**	**B**	**B**
Xtxp57	6	141	**A**	**B**	**B**	**B**	**A**	**B**	**B**	**B**	**B**	**B**
Xtxp10	**9**	67.2	**A**	**B**	**B**	**A**	**B**	**B**	NA	**A**	NA	NA
Xtxp67		**87.9**	**A**	**B**	**B**	**H**	**B**	**B**	**B**	**H**	**B**	**B**
Xtxp410		**115.4**	**A**	**B**	NA	**A**	**B**	**B**	**B**	**H**	**B**	**B**
Xtxp358		126	**A**	**B**	**A**	**H**	**B**	**H**	**B**	**H**	**B**	**A**

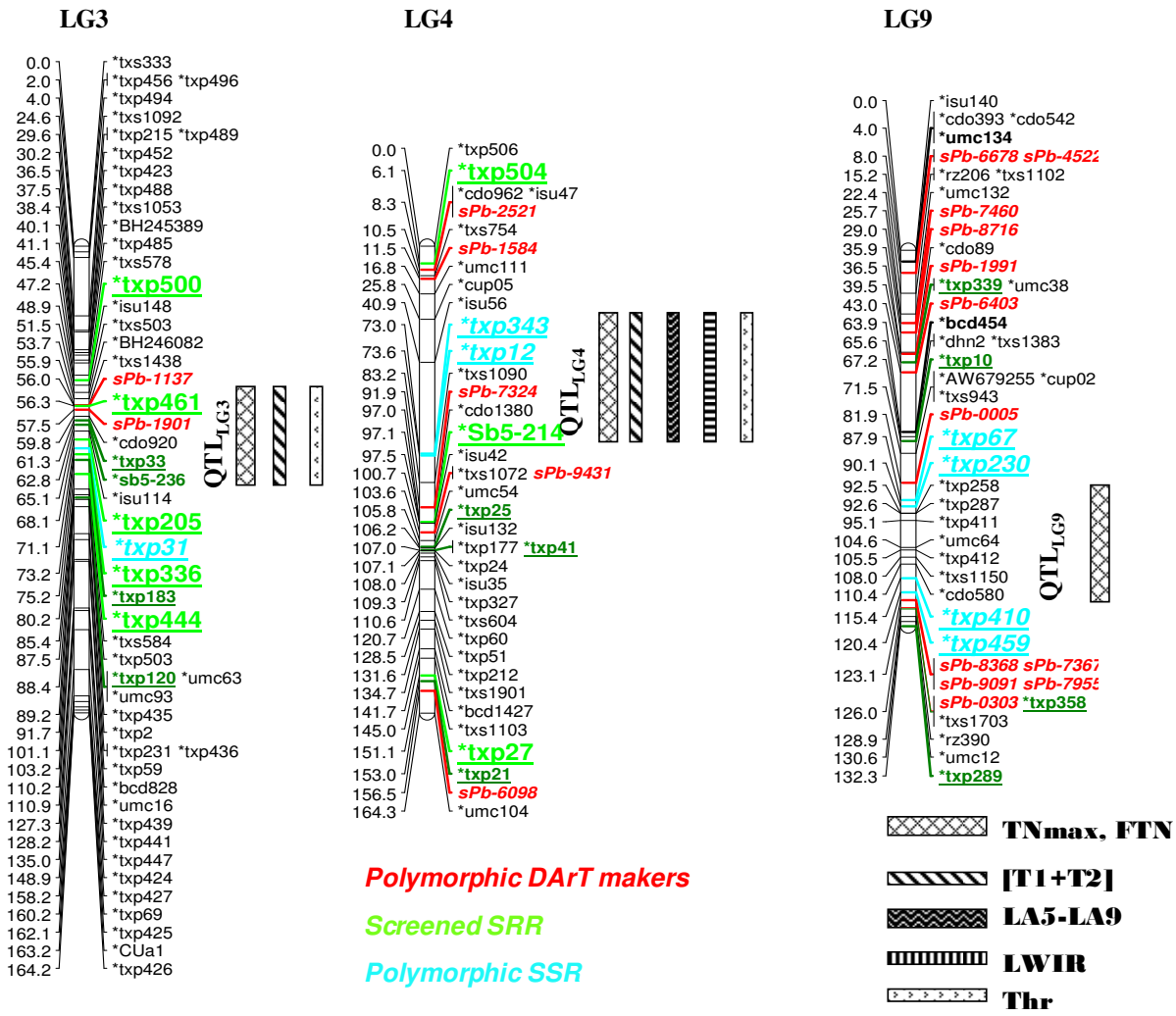


Figure 6: Localisation of three putative QTLs linked with tiller number (TNmax, FTN) and its key component traits ([T1+T2], LA5-LA9, LWIR, Thr) on LG3, 4 and 9 in BC2F2 populations genotyped with SSR markers.

Association of SSR markers with tillering components

The four polymorphic SSR's were all significantly associated (LOD values over 3.0) with tiller number, including both TNmax and FTN. However, among specific tiller rank components (T1 to T6), only early tiller ranks [T1+T2] was consistently associated with Xtxp31 (LG3) and Xtxp12 (LG4) across populations (Fig. 6).

Considering only populations where the selected markers were segregating,

parameters associated with leaf size (individual leaf area (LA5 to LA9), individual leaf width change, LWIR) were associated with Xtxp12 (LG4) only. By contrast, the threshold for tillering (thr) was associated with both Xtxp12 (LG4) and Xtxp31 (LG3) (Fig. 6). For markers on LG9, there was no clear association across populations.

The other traits, e.g. main stem total leaf number, days to anthesis and plant height, did not show any significant association with the screened SSRs (Xtxp31, Xtxp12, Xtxp67 and Xtxp410).

DISCUSSION

Successful exploitation of genetic information and genomics tools in breeding programs requires extensive and precise phenotyping of traits of interest in breeding materials or mapping populations (Varshney *et al.*, 2005)..This study was conducted to identify QTLs associated with tillering in sorghum and to illustrate how ecophysiological modelling concepts integrating knowledge of the underlying processes can be used as a tool to assist genetic studies (Hammer *et al.*, 2005; Yin *et al.*, 1999). As a preliminary step, Kim *et al.* (2008a,b) dissected tillering into underlying component traits, associated with crop growth and development and developed a generic framework in which tillering was controlled by the supply/demand status of the crop and coordinated with main stem leaf development. Under plant internal competition for carbohydrate, key environmental and genetic components regulating tillering dynamics were identified. In this paper, we extend this framework through the incorporation of QTLs.

Identification of three putative QTLs related to tillering

Three QTLs related to tiller number (both TNmax and FTN) were identified, localised on LG3, 4 and 9 (identified as QTL_{LG3}, QTL_{LG4} and QTL_{LG9}) based on associations with SSR genotyping in the BC2F2 populations and DArT genotyping of BC1F4 parental

lines (Fig. 6). Tiller distribution among each BC2F2 population (Fig. 2) was in accordance with the tillering behaviour of the BC1F4 parental lines, i.e. populations derived from high tillering inbred lines had the widest range of variation (F2_R05422, F2_R05429 and F2_R05430). Individual tiller rank frequencies among population (Fig. 3) also confirmed earlier results (Kim *et al.* 2008b) that the major difference between high- and low-tillering lines was associated with differences in the appearance frequency of early tiller ranks [T1+T2]. This frequency was indeed consistently associated with QTL_{LG3} and QTL_{LG4}, whereas the other tiller ranks showed no association.

Correspondence with other identified QTL or genes

The QTLs identified in this study corresponded with those found in a number of previous studies. The QTL on LG3 is closely associated with a QTL for stay-green reported by Crasta *et al.* (1999). Stay-green reflects the ability to retain green leaf area under drought stress, and tillering is important in this respect, as it can affect leaf area dynamics and hence the temporal pattern of water use (Hammer, 1996). The QTL on LG3 affected tillering through the frequency of appearance of lower-rank tillers, and this has been identified as an important mechanism of drought adaptation (van Oosterom *et al.*, 2008). The QTL on LG4 co-located with a QTL for plant height identified by Klein *et al.* (2001). The QTL on LG9 also co-located with a QTL for height identified by Lin *et al.* (1995). While no associations were found with height in this study, this finding is consistent with observations from near-isogenic lines on a linkage between plant height and tillering (George-Jaeggli, pers. comm.) that is known to be connected with effects on polar transport of auxin (Multani *et al.*, 2003). Although the location of that major height gene differs from the locations identified here, the effect might be associated with modifiers affecting panicle length as, in this study, height was measured to flag leaf ligule (not total plant height), and the regions found co-locate with QTLs for panicle length (Hart *et al.*, 2001).

Association of QTLs with plant internal competition model framework

Mapping QTLs that control variation in traits of agronomic importance is a key part of the process of using molecular markers in breeding programs. However, many QTLs are either environment or population dependent and for most QTLs (or cloned genes) the underlying physiological mechanism remains unknown. In this work, we used a model framework to phenotype key component traits related to tillering dynamics, using a framework relating tillering to the internal competition status (S/D index). Early tiller ranks [T1+T2] characterised the main impact of early assimilate availability resulting from difference in leaf morphogenesis on the main stem, captured by LWIR in the model equation and a threshold value (Thr). LWIR was more consistently related to QTLs than the absolute leaf width of a specific leaf rank, suggesting a more direct genetic control of LWIR. Genotypic differences in LWIR could represent differences in the number of cells rows and hence meristem size.

There was a significant negative correlation between LWIR and tiller number for four of the BC2F2 populations and interestingly, the BC1F4 parental lines had indeed *S. arundinaceum* allele for the considered SSR markers (Xtxp31 and Xtxp12, Table 4). Those phenotypic and genotypic correlations support the hypothesis that tiller number is causally related to LWIR (i.e. leaf morphogenetic characteristics), although other mechanisms involving other QTLs can be occurring in other populations.

Xtxp12 is strongly associated with a QTL related to tillering through overall leaf size (LA5-LA9), in particular through LWIR. Xtxp31 connects to early tillering dynamics [T1+T2] via Thr rather than directly through overall leaf size, and it seems to be connected to a threshold internal competition value for tiller emergence.

In summary, hypotheses about the putative QTLs functions based on their association to our model parameters and key elementary components can be suggested as in Fig. 7. QTL_{LG4} would be directly responsible for LWIR and Thr, thus having subsequent effect on

individual leaf area and early tillering, whereas QTL_{LG3} seem to control a threshold value for internal competition and have an effect on early tillering. Specific function of QTL_{LG9} and how the underlying genes would impact tiller number could not be defined in this study.

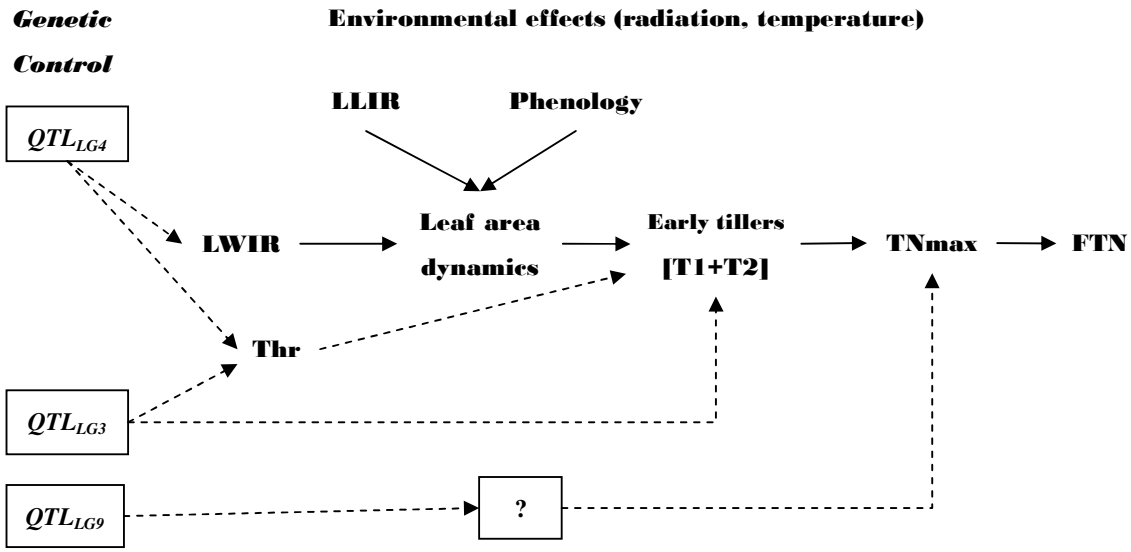


Figure 7: Hypothetic QTLs actions on model-based coefficients and component traits underlying tillering dynamics

Genetic control of leaf development and final organ size

A number of genes have been identified as determining leaf development and final organ size through modification of cell division in *Arabidopsis*. These genes affect organ size in all dimensions but some were implicated particularly in the determination of leaf width, such as the gene encoding G-protein β subunit (Lease *et al.*, 2001), *AINTEGUMENTA* (*ANT*) regulating cell number during organogenesis (Mizukami and Fischer, 2000) and *ARGOS* affecting organ size in the lateral plane (Hu *et al.*, 2003). Tsuge *et al.* (1996) proposed leaf expansion involves at least two independent developmental processes: width development and length development, with the *ANGUSTIFOLIA* and *ROTUNDIFOLIA3* genes playing different polarity-specific roles in cell elongation. In maize, level of expression of gene

encoding p34cdc2 kinase in leaves was related to cell division (Granier *et al.*, 2000). However, no connection was made between genetic determination of organ size and specific ability with branching ability. With the availability of the complete sorghum sequence, it will be possible to have a candidate gene approach for the QTL associated with Xtxp12 for example.

Results support the concept of model-based phenotyping for complex traits

This study provided a test of the concept of using model-based approaches to genetic analysis of complex traits. The QTLs for the component traits co-located with 2 of the 3 QTLs identified for tillering directly (fig. 6). Further the physiological dissection provided by the model-based phenotyping can be used to consider putative function of the relevant regions as differing aspects (LWIR and Thr) aligned with the two co-located QTLs. The absence of alignment with the third tillering QTL suggests another mechanism not included in the current modelling framework. Even though tillering is relatively straightforward to phenotype, as it is a highly visible phenotypic trait and GxE interactions appear minor (Kim et al 2008b), this co-location outcome nonetheless supports the notion that model-based approaches can aid with genetic analyses of less visible complex phenotypic traits (where the potential to test for co-location would not be possible or as straightforward).

Even though tillering is relatively straightforward to phenotype, the model-based approach provides some direct benefit as the component traits related to leaf morphogenesis, such as LWIR, are constitutive and genotype-dependent and so less prone to the known environmental variations. The detection of QTLs of parameters such as LWIR could be undertaken in young plants in an environment not conducive to tillering. Further, such QTL associations with physiological determinants can be used to link the genetic basis of sorghum tillering with morphogenesis in existing crop model platforms, such as Ecomeristem (Luquet *et al.*, 2006) and APSIM (Wang *et al.*, 2002). This would allow simulation of QTL effects on tillering and associated consequences on crop growth and yield.

CONCLUSION

In this study we have identified three genomic regions associated with tillering in sorghum by phenotyping relevant populations directly for tillering. By also phenotyping those populations for attributes derived from a modelling framework for tillering developed earlier, we have tested the concept of using model-based approaches to enhance genetic analysis of complex traits. The co-location of QTLs found illustrated how a modelling framework could potentially assist genetic studies of complex traits in a manner relevant to breeding programs.

"Let my teaching fall like rain
and my words descend like dew,
like showers on new grass,
like abundant rain on tender plants."

"My heart is blighted and withered like grass;
I forget to eat my food."

“When evening comes, you say,
It will be fair weather, for the sky is red,
and in the morning,
Today it will be stormy, for the sky is red and overcast.
You know how to interpret the appearance of the sky,
but you cannot interpret the signs of the times.”

CHAPTER V

Modelling concepts for tillering control by competition for carbohydrates: proof of concept and simulation with *EcoMeristem* model

Hae Koo Kim
Delphine Luquet
Anne Clément-Vidal
Michael Dingkuhn

CHAPTER V

INTRODUCTION

Crop modelling developed since the early 1970s (de Wit, 1970) was initially viewed as a tool to describe and predict phenomena at one level of biological organization (plant or crop) by integrating responses at a lower explanatory level (organ or plant). Quantitative descriptions were the basis to explore theories and explain how plants and crops behave, taking into account the interaction between components such as growth and development. Environmental effects on resource acquisition processes (photosynthesis, water and sometimes N uptake) are the main drivers of crop growth and yield in the most current crop models, based on developmental rules (e.g. phenological phases controlling leaf area development, flowering time) and resource partitioning patterns among organs considered to be largely independent of resources. Processes determining assimilate demand in the plant and its regulation by environment (E) and genotype (G) were thus not simulated in most cases (Dingkuhn *et al.*, 2005).

Recently, sorghum tillering was addressed as a important, complex, component trait for grain yield that is subject to regulation by E and G (Kim *et al*., 2008a,b). In these studies, five experiments were carried out across a wide range of photo-thermal conditions (incident irradiance per developmental unit) using contrasting sorghum genotypes in terms of tillering (MR Buster, and five hybrids from the same BC1F4 from an initial cross between a low tillering, high yielding elite hybrid 'R931945-2-2' (recurrent parent) and a high tillering wild type, *Sorghum arundinaceum*). Resulting modelling concepts stated that tillering is controlled by plant internal competition for carbohydrate (C), expressed as a single equation computing C supply by demand ratio (named S/D index). S/D index captured both E and G effects on sorghum tillering. A conceptual model framework was built on a detailed analysis of the competition for C among sinks (new or expanding organs) in the course of morphogenesis, depending on organ size and expansion rate. Plant internal competition for C between main stem and tillers was related to main stem successive leaf width increase rate (LWIR). LWIR and leaf size were good indicators of genotypic differences in tillering potential in a given environment, probably because they were indicative of demand for C. Environment mainly played on plant internal competition for C through its impact on leaf length (characterized by main stem leaf length increase rate, LLIR). In addition, it was shown that tillering variability across G and E levels was mainly explained by the difference in the frequency of early tiller occurrence, possibly indicating the importance of competition processes occurring at pre-tillering phase.

Such model concepts were thereafter applied to phenotype eight BC2F2 populations derived from a second backcross between the recurrent parent and respectively eight (high or low tillering) lines of the BC1F4 population form. Based on a single experiment (one location), genotypic differences in tillering were related to genotypic variation in S/D index. This enabled identifying putative QTL for elemental processes of tillering (component traits), presumably connected to a smaller number of genes and less prone to G x E (Kim *et al.*, 2008c).

The fact that the hypotheses of S/D dependency of tillering, initially derived from a small number of genotypes, was also confirmed on mapping populations encouraged us apply this concept to quantitative modelling. Specifically, the question is whether this concept can be used to upgrade two existing crop models dealing with sorghum morphogenesis: *EcoMeristem* (Luquet *et al.*, 2006a) and APSIM (Wang *et al.*, 2002).

The present study aims at (1) providing further proof of concept for the model of sorghum tillering control by plant internal competition for C, and (2) to test the concept in the context of quantitative, whole-plant modelling. For this purpose, a complementary study was carried out to characterize root to shoot mass partitioning and sugar (soluble and starch) distribution among organs under competition for C in shoots, on three of the six contrasting hybrids in terms of tillering already studied by Kim et al. (2008b). Competition for C was thereby attenuated by shading a treatment. Preliminary tests were then conducted of a modified version of the *EcoMeristem* model implementing the concepts developed on tilllering control, using data presented by Kim *et al.* (2008a,b).

MATERIAL AND METHODS

Experiment

In Kim *et al.* (2008a,b), five experiments were carried out between 2005 and 2006 in three open field and two controlled environments that generated contrasting photo-thermal conditions and contrasting tillering behaviour among six genotypes (MR buster and five hybrids). In each study plant vegetative phenology (main stem and tiller leaf appearance, expansion and ligulation rate) was described, while plant leaf area (PLA) and dry weight per organ type (green leaf, dead leaf, stem for each culm) was harvested at different stages of vegetative development until anthesis (pre-tillering stage, during tiller emergence phase, end of tillering phase, flag leaf stage and anthesis).

A complementary experiment was carried out in field conditions in Montpellier (sown on 2 August 2006, 43°38'N, 3°52'E, 46 m asl), to explore the carbohydrate (C) distribution and concentration among plant organs, considering two treatments: full sunlight (S) and shaded (Sh); for the latter, plants were covered (at about 1 meter above the canopy) with a filet attenuating incident irradiance by 35%. Three representative hybrids of those studied by Kim et al. (200Bb) were selected among previously studied hybrids (R931945-2-2 designated as LT, R999100 designated as HT and Buster). It was chosen to apply a weak level of light attenuation in order to minimize the potential effect of light intensity reduction on leaf size (generally showing increased length under such conditions). The experiment was a split-split-plot design with three replications, two treatments and three genotypes while a sub-treatment was considered as the sampling hour at a given date (one sub-sampling was realized soon after sunrise to evaluate sugar content after the night, while a second sub-sampling was realized in the middle of the afternoon after photosynthetic activity reached its maximal level). Two harvests were realized, the first around leaf 3-4 fully expanded stage, i.e. at pre-tillering stage, and the second at leaf 6-7 fully expanded stage, i.e. during tillering stage. Here we only focus on the stage prior to tillering as sorghum shoot fly invasions (main shoot killed) did not leave enough plants for a second harvest. For each harvest, leaf number (ligulated or expanding) on the main stem was counted, main stem last ligulated leaf area was estimated by measuring blade length and maximum width (sheath length was also measured); plant organs were then separated into different compartments: the last ligulated leaf considered as a source leaf (leaf 3 and leaf 4 were systematically separately sampled for the first sampling), whole plant sheaths, the rest of the blades (except leaf 3 and 4) and roots (thereafter cleaned to remove all soil particles). Each sample was used for both dry weight estimation (dw) and sugar content analysis (except for roots): water soluble carbohydrate: glucose, fructose and sucrose, and reserve sugar: starch). Also, the specific leaf area (SLA, $cm^2.g^{-1}$) of leaves 3 and 4 on the main stem could be estimated as the ratio between corresponding LA and dw. The method used for sugar content analysis, based on High Performance Liquid Chromatography (HPLC), was described in detail by (Luquet *et al.*, 2005a; Luquet *et al.*, 2006a).

***EcoMeristem* model**

The model *EcoMeristem* was recently developed to simulate and analyze cereals morphogenesis under genetic and environmental control, based on a formalization of main phenotypic plasticity processes. The model was developed using rice as a model plant for cereals, focusing first on vegetative stages. Also some of the initial model concepts were very

specific to rice. It was described in detail by Luquet *et al.* (Luquet *et al.*, 2006a); (Dingkuhn *et al.*, 2006). We provide here a description of its main modelling concepts and its last improvements.

EcoMeristem is a crop model dynamically simulating whole (average) plant morphogenesis and its potential phenotypic plasticity depending on plant internal competition for C (C source, computed at crop level) among sinks (C demand of expanding organs and new organs to be created) based on a simplified formalization of meristem behaviour. The model relies on two key concepts:

a- Organ initiation rate, scheduled by plastochron (equal to phyllochron for rice and defined by *phyllo,* genotype dependent parameter): once initiated, an organ *n* is pre-dimensioned according to the size (length, width) of the previous leaf *n-1* and the *MGR* (Meristem Growth Rate, genotype dependent parameter), coefficient added to final leaf *(n-1)* size. Once pre-dimensioned a leaf begins to expand, with a Leaf Expansion Rate (LER, $cm^2.°d^{-1}$) equal to the ratio between final leaf length and expansion duration (assumed to be the plastochron or the phyllochon in the model, as both are equal for rice). The sum of daily leaf expansions to be accomplished across leaves in the plant corresponds to plant daily demand for carbon. It is translated in terms of dry weight (i.e. C) using a leaf rank dependent Specific Leaf Area (SLA) logarithmic function detailed by Luquet et al. (2006) and relying on one key parameter, *SLAp*, defining the degree of SLA decrease from one leaf rank to the successive one on the same axis. It must be mentioned that a simulation begins at plant germination based on two key genotype dependent initial parameters: grain dry weight and first leaf size (length and width), leaf already present in the embryo and considered as a strong genotypic parameter (Condon *et al.*, 2004). Potential time for tiller outgrowth is scheduled by phyllochron, i.e. by leaf appearance on the main stem. One tiller can be created per existing stem constituted at least by four leaves. The size of the first leaf on a new tiller is defined by the average between main stem leaf 1 and current expanding leaf. Thereafter, leaf size on a given tiller is pre-dimensioned as on the main stem.

Root growth rate on day i is proportional to shoot growth rate on day (i-1), using an empirical function decreasing exponentially from germination until flowering (Luquet *et al.*, 2007).

Nb: in its previous version, organ dimensioning in EcoMeristem was based on organ dry weight and not dimension (Luquet et al 2006, 2007). As detailed below, this was modified as most of phenotypic plasticity processes play first on organ size rather than weight.

b- A plant internal competition index *Ic* is daily computed as $Ic=S.D^{-1}$, S being C supply (assimilates available to the plant according to external resources and plant leaf area, close to soluble sugar role) and D being C demand (sum of daily individual organ demand for growth). Depending on *Ic*, potential organ number and size is regulated each time the plant reaches a new plastochron:

- If $Ic < 1$, the potential size of any organ being initiated is down-regulated;
- If $Ic < Ict$ (*Ic* threshold value for tillering, genotype dependent parameter) then tillers are not created; otherwise, the potential number of tiller is created (Luquet *et al.*, 2007).

At a given day of plant growth (and organ expansion), if $Ic>1$, excess carbon is stored in a reserve compartment (comparable to starch role in the plant), or, if the storage compartment is saturated, the assimilation rate is reduced by feedback. By contrast, if $Ic<1$, reserves are mobilized; the oldest leaves are 'killed', followed by reallocation of some of their biomass.

c- Environment modules: Resource (water, light) acquisition and S computation used in *EcoMeristem* are today very simple and considered at crop level. Regarding S computation, plant leaf area is daily computed at population level to apply the Beer Lambert law and compute plant net CO_2 assimilation based on radiation use efficiency (Dingkuhn *et al.*, 2003). A water balance module was recently implemented but won't be detailed here as the presented study only considers non limiting water conditions.

New concepts for modelling tillering control by C availability:

Sorghum tillering modelling framework was design to simulate sorghum tillering as a dynamic trait controlled by plant internal competition for C (estimated through a S/D index) depending on E and G, and anchored in main stem development and growth processes (Figure 1). It provides formalisms to be used to improve the way tillering (of sorghum and cereals more generally) is accounted for in existing models, particularly in *EcoMeristem*. However, only some of the concepts presented here below were already implemented in *EcoMeristem* model, implying that only preliminary results are presented.

a- Phenology: main stem tip and ligule phyllochron

The detailed analyses of tillering pattern from its initiation and outgrowth to its senescence or fertility put emphasis on the coordination of tillering processes with main stem development and growth (Kim et al. 2008). An important particularity for sorghum is that leaf tip and ligule appearance rate (respectively designated as tip-phyllochron and lig-phyllochron)

of each axis are not equal (by contrast with rice) during the vegetative phase. Tip-phyllochron is shorter than lig-phyllochron thus the duration of expansion of each successive leaf is increasing with leaf rank

$$(LED_{Lrank} = [\text{lig-phyllochron} - \text{tip-phyllochron}] \times L_{rank}) \quad [1]$$

Therefore at the end of vegetative stage, up to three to four leaves are expanding on a given axis simultaneously (while there is only one expanding leaf at a time in the case of rice).

Overall, there was a very robust coordination between main stem leaf development and a specific tiller emergence (Kim *et al.*, 2008a). In other words, successive tiller rank (both in terms of initiation and appearance) could be correlated with a specific main stem leaf rank, for example T1 and main stem leaf 3, T2 and leaf 4 and so on. However, for non fertile tillers the effective start of outgrowth seems to be delayed up to one leaf rank compared to such coordination. The coordination between main stem leaf rank and tiller rank was confirmed across all field experiments but in controlled environments and in 2006 complementary experiment (with either low radiation or very high temperature conditions), there was a shift of one leaf rank to this general framework (Fig. 1).

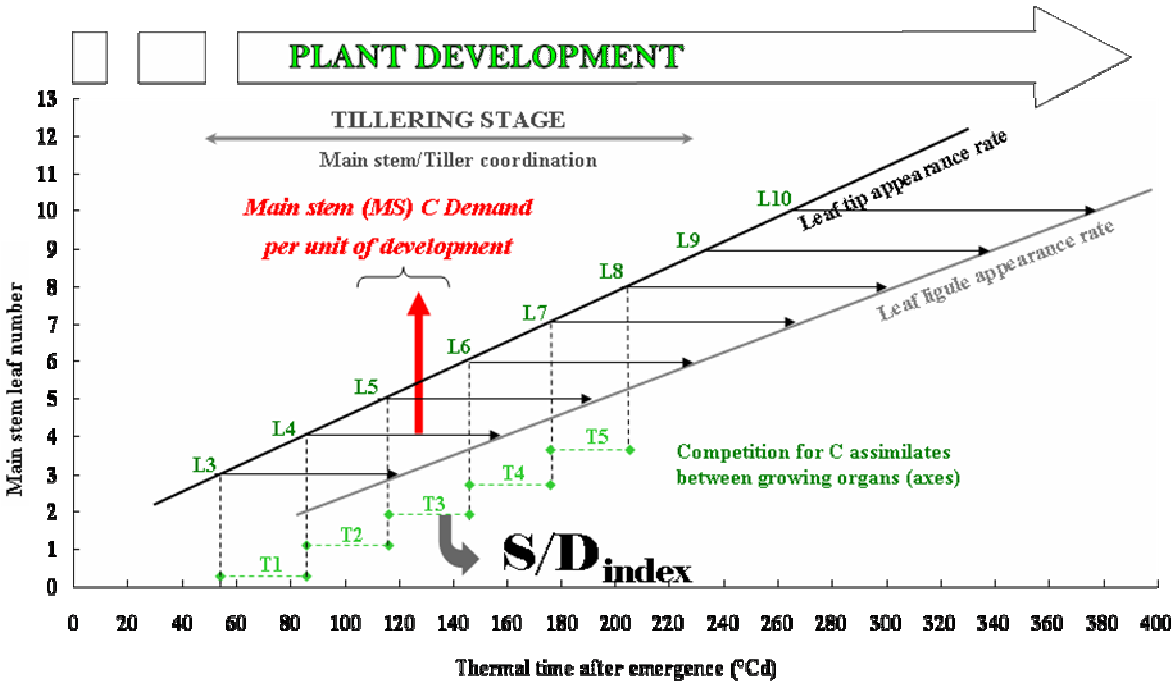


Figure 1 : Tillering generic framework based on main stem development and growth with successive tillers optimum window of emergence opportunities.

Dynamic framework for the genotypic and environmental control of tillering

Our framework assumes that a surplus of C assimilates would allow an axillary bud to grow out as a tiller, provided this occurs within a window of potential appearance in relation to environmental conditions and apical dominance (Kim *et al.*, 2008b). Similarly, fertility or senescence of emerged tillers would be driven by assimilate availability at the whole plant level and related to sink to source process regulation (Dun *et al.*, 2006; Luquet *et al.*, 2006b). If C assimilate surplus condition was maintained, all axes would continue to grow to the potential and the excess would constitute reserves into leaves (SLA lower) and stem compartments (Lafarge and Hammer, 2002). If C demand exceeds supply, priority would be given to the strongest sink, i.e. the axis with the most advanced growth, causing the weakest sink to cease development and growth. Sink strength is determined by tiller order, and by the timing of tiller appearance within the tillering time window: the earlier a tiller outgrows within its time window, the more competitive it is relative to upcoming axes. As the outgrowth of T1 and T2 is usually delayed within the tillering window (Bos and Neuteboom, 1998), these tillers are in a competitively disadvantaged position compared to T3 and consequently often cease growing and become non-productive. Overall this leads to the observed tiller hierarchy, where T3>T4>T5+ as well as T3>T2>T1 (Kim et al., 2008a; Lafarge *et al.*, 2002). This concept is consistent with Dusserre *et al.* (2002), who demonstrated that cotton plants do not only adjust final organ size to the level of competition among assimilate sinks, but also adjust organogenesis and the period of organ expansion.

b- Tillering control by S/D status

The coordination between the effective outgrowth of a tiller rank with the development of a given leaf on the main stem is shown in Fig.1. A specific tiller rank emergence can thus occur within a time windows of emergence opportunity; e.g. T3 outgrowth starts between main stem L5 and L6 appearance (Figure 1). However, the earlier a given tiller emerges within its window of appearance, the more competitive it will be in terms of C compared to other expanding organs (which can explain why a tiller with a delayed emergence is usually non fertile). Therefore a given tiller rank appearance frequency at the canopy level (frequency of appearance of T_x) depends on the plant internal competition level during the defined window of emergence opportunity, that can be estimated by the S/D index (Eq. 2, illustrated by the case of T3 in competition with main stem leaf 5 expansion). S is a function of E (RAD, irradiance level per unit of development during the considered developmental phase, that is, leaf 5 expansion duration LED_5) and G (plant leaf area at the considered time, represented here by main stem leaf 5 area, LA_{L5}); D depends on the size of the leaf in competition with tillering on the main stem during the considered period, and is

also function of E (because of E effect on leaf length, here defined by main stem LLIR) and G (characterized by main stem LWIR).

$$S/D_{index} = \frac{RAD_{LED5} \times LA_{L5} \times DevPhase}{TT_{LED5} \times LLIR \times LWIR} \quad [2]$$

c- Allometries

In controlled environment experiments Exp4 and Exp5 in Kim *et al.* (2008b), root biomass was measured to calculate a root/shoot ratio at pre-tillering stage and during tillering phase; no genotypic difference could be shown, which was confirmed in the 2006 complementary experiment. Therefore, root compartment demand for C was considered as similar across genotypes and environments, which is however a strong simplification.

Leaf sheath vs. blade ratio in terms of dry weight and length was also investigated but did not show any significant difference between genotypes although there were some environmental effects on partitioning.

d- Implementations in *EcoMeristem*

In a first step, *EcoMeristem* was upgraded by differentiating leaf tip and ligule phyllochron as suggested in Eq.1, and using the allometries experimentally defined in (c). However:

- tiller potential outgrowth scheduling was kept as coordinated with the appearance of a leaf at a given rank on the main stem (no time window was defined);
- plant internal competition for C was kept as estimated by Ic and mainly controlled by MGR (leaf dimensioning) and phyllochrons (largely controlling expansion duration).
- tiller response to competition for C was still controlled by Ict parameter (genotypic threshold of response to Ic).

Statistical analyses

ANOVA and other statistical analyses (correlation matrices and multiple regressions) were computed with either Statbox6.5 (Grimmersoft, Paris, France) or R (R Development Core Team, 2007)

Proof of concept: Experimental observations on carbohydrate levels

ANOVA tests were performed on all sugar and morphogenetic variables studied and results are summarised in Table 1. Light treatment (E effects) and sampling hour had major effects on sugar concentration whereas genotype effects were small or absent, depending on the variable measured. Specific leaf area (SLA) was strongly affected by all factors.

Table 1 : ANOVA results regarding genotype, treatment and sampling hour effects on plant organ sugar concentrations (glu: glucose, fru: fructose, suc: sucrose ans starc) and dry weight (dw), leaf area (LA) and specific leaf area (SLA). Significance levels: (.) at p<0.1; (*) at p<0.05; (**) at p<0.01 and (***) at p<0.001.

	Leaf 3									Sheath					
Effects	[Glu]	[Fru]	[Sucrose]	[Starch]	dw	[Total sugar]	LA3	SLA3	SLAstruc3	[Glu]	[Fru]	[Sucrose]	[Starch]	dw	[Total sugar]
Line	.	.		*				***	***						
Treatment	*		**			*		***	***	*		*	***	*	***
Time	***	***	***	***	***	***		***	***	*	**	***	***		***
Line*Treatment								*	*						
Line*Time	*			.				*	*						
Treatment*Time	**	*	**	*		**						*			
Line*Treatment*Time	**	.				.			*			*			
	Leaf 4									Remaining leaves					
Effects	[Glu]	[Fru]	[Sucrose]	[Starch]	dw	[Total sugar]	LAI4	SLA4	SLAstruc4	[Glu]	[Fru]	[Sucrose]	[Starch]	dw	[Total sugar]
Line															
Treatment	*	*	*					**	**		*		.	**	
Time	**	*	***	***	**	***		***		.	***	***	***	.	***
Line*Treatment															
Line*Time															
Treatment*Time			**	***		***				*			*		.
Line*Treatment*Time															

a- Environment effects

The impact of light level (E) on sugar accumulation in the plant was first analysed for shoot total sugar concentration (soluble sugars and starch in all shoot organs) (Figure 2) For all genotypes in the afternoon (PM), plants in S treatment accumulated significantly more C than in Sh treatment (P<0.05 in Table 1). No E effect could be observed in the morning (AM) on plant total sugar concentration. Its increase from AM to PM was therefore reduced under Sh treatment.

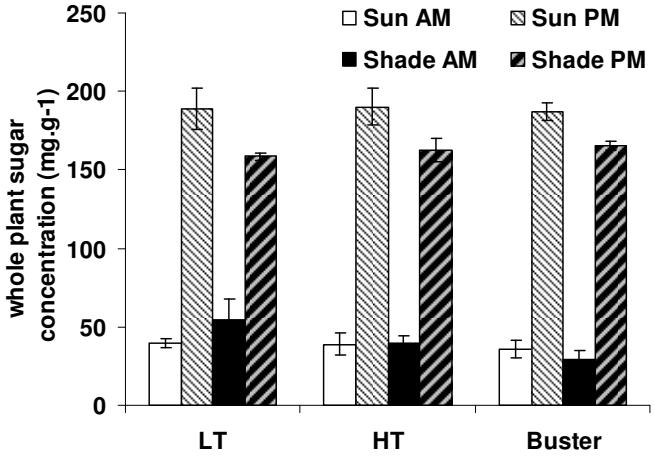
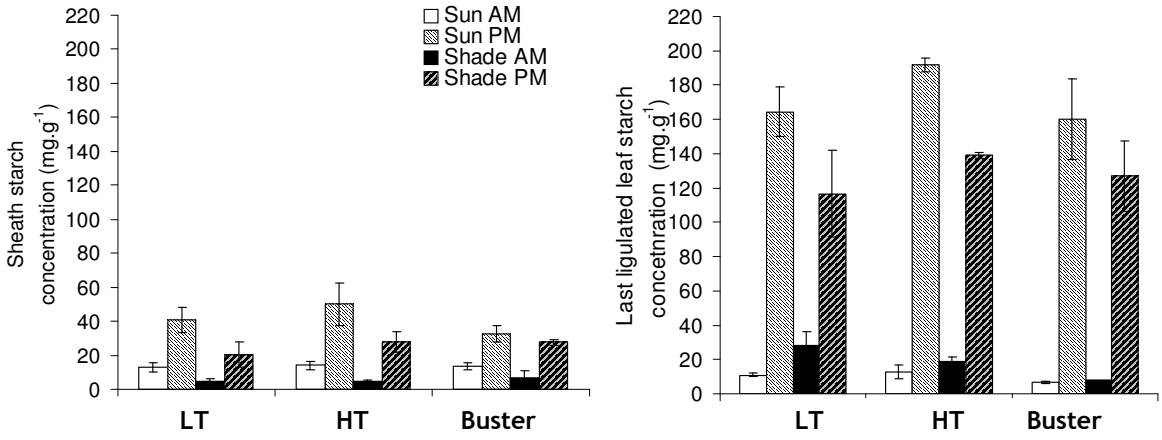


Figure 2: Plant shoot total sugar concentration of genotypes LT, HT and Buster, in Sun and Shade treatments, at morning and afternoon sub-samplings.

Starch and sucrose, taken individually in sheaths (left graphs in Fig. 3) and source leaves (represented by leaf 4 in right graphs of Fig. 3), show generally a reduced concentration at PM under Sh compared to S conditions. Similar to shoot total sugar concentrations, starch and sucrose concentrations at AM did not show any E (shade) effect while the increase between AM and PM was maintained but attenuated in Sh compared to S treatment. However, E effects on sucrose and starch at PM were not statistically significant ($P>0.05$, see Table 1) to the exception of starch in sheaths and sucrose in leaf 4 ($P < 0.05$ in Table 1). The contribution of glucose and fructose to E effects on shoot total sugar concentration was small, first because the hexoses represented only small fractions of total, as compared with sucrose or starch (results not shown); and second because glucose and fructose concentrations were constant (sheaths) or even slightly increased (leaf 4) at PM under Sh treatment. Fructose concentration showed generally the same behaviour as glucose but with a ratio of 0.5 to 0.75, depending on organ type.



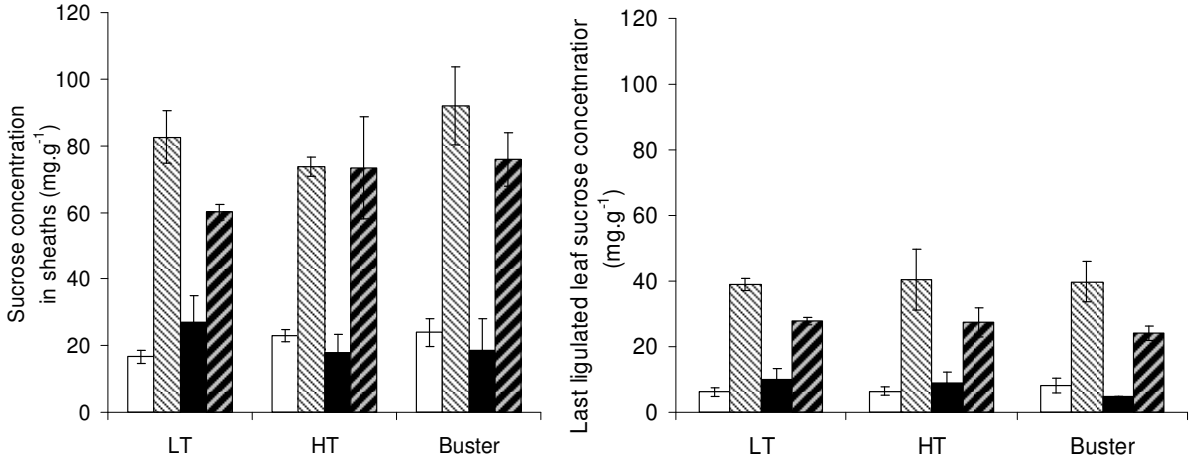


Figure 3 : sheath (left) and leaf 4 (right) starch (top) and sucrose (bottom) concentrations for LT hybrid, HT hybrid and Buster in Sun and Shaded treatments at morning (AM) and afternoon (PM) sub-samplings.

Regarding morphogenetic variables, no E effect could be observed on plant developmental rate in terms of leaf appearance rate, and the three genotypes had the same phyllochron as already observed across experiments in Kim *et al.* (2008b; results not shown). This enabled sampling plants on the same day for the targeted developmental stage (i.e. pre-tillering stage when leaf 3 or 4 is ligulated).

Significant E effects could be observed on both the last ligulated leaf SLA and structural SLA (noted SLAstruc, estimated by dividing LA by dw with non-structural carbohydrates subtracted). This is presented in Figure 4 where both SLA and SLAstruc of leaf 4 show significantly higher value under Sh compared to S conditions ($P<0.05$ in Tab.1. By contrast, no significant E effect could be observed on final leaf 3 and 4 area ($P>0.05$ in Tab.1), although Buster showed numerically larger leaf area under Sh treatment (Figure 4 for leaf 4).

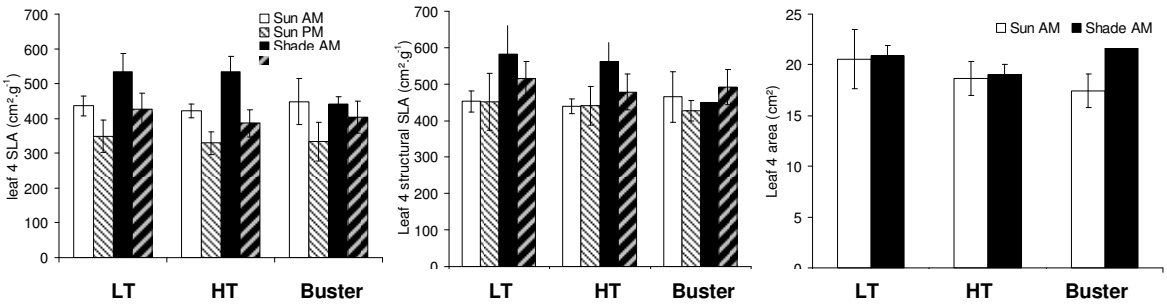


Figure 4 : SLA (left), SLAstruc (middle) and area (right) of leaf blade 4 of LT, HT hybrids and Buster, under Sun and Shaded conditions at morning (AM) and afternoon (PM) sub-samplings (for SLA and SLA struc) and in average on both sub-samplings for leaf area.

b- Genotype effects

No significant genotypic effects could be observed on sugar and morphogenetic variables, to the exception of SLAstruc and SLA of leaf 3 ($P<0.05$ in Tab.1). In particular, average (on AM and PM) leaf 3 SLAstruc in S treatment was greater for HT (435.7 +/- 11.5 $cm^2.g^{-1}$) compared to LT and Buster, respectively at 413.3 +/- 25.3 and 390 +/- 32 $cm^2.g^{-1}$. The three genotypes showed similar plant shoot total sugar concentration under S and under Sh treatments (Figure 2 and Tab.1, $P>0.05$). However, Figure 3 shows that HT hybrid had generally higher starch concentration in both sheaths and leaf 4 under S and Sh treatments compared to other genotypes (whereas no difference could be observed in terms of soluble sugars, i.e. sucrose in Fig. 3 or glucose, not shown). Finally, Figure 4 shows that under S conditions, leaf 4 area was larger (but not significantly, see Tab.1) for LT (average of 20.75cm^2) than HT and Buster (respectively 18.2 and 17.4cm^2). Under Sh conditions, Buster was the only genotype showing E effect (e.g. for leaf 4 in Figure 4).

c- Sugar availability and distribution vs. morphogenesis across G and E levels

In the experiment on plant sugar content, environments more or less favourable to tillering were generated by comparing a full light treatment S with a shading treatment Sh (light attenuation by 35%). Genotypes showed a reduction od shoot total sugar concentration reduction under Sh at PM but no clear genotypic difference was observed for sugar or morphological variables, to the exception of (i) higher starch concentration in the afternoon for HT hybrid under both Sh and S treatments, (ii) larger leaves for LT hybrid in S treatment, and (iii) an E (Sh) effect on leaf size only for Buster.

Shade (E) and sampling hour (H) effects could be clearly observed on sugar concentrations, SLA and (for E only) on SLAstruc. There is thus evidence that sugar concentration in the leaf reduces SLA (i.e., increases leaf weight). However, it is difficult to evaluate the contribution of sugars compared to other components (i.e. other mobile substances or structural mass) to variation of SLA depending on E and H. In order to quantify this phenomenon, leaf 4 dw per unit area, i.e. SLW (specific leaf weight in $g.cm^{-2}$) was computed and its variation (in percent) across E and H compared to that of leaf 4 total sugar concentration (Table 2). In terms of H effects, SLW increase from AM to PM was explained

by 65% (Sh) to 90% (S) by variation in sugar concentration. On the other hand, regarding E effect for a given level of H, the reduction of leaf SLW of 10 to 20% from S to Sh treatment was not at all (AM) or to a minor extent (PM) explained by sugar concentration. Consequently, the effect of E on SLA (or its reciprocal, SLW) is not brought about by variable sugar concentration and thus represents variation in structural mass. This does not exclude, however, indirect effects of light on leaf mass *via* sugar availability(assimilate source), resulting in leaf structure adjustment (sink) to the limiting resource. This is supported by the results on SLAstruc (Figure 4), showing that leaf structural mass was reduced in Sh treatment. However, it is possible that other non-structural leaf chemical components not quantified in this study contribute to SLA variation.

Table 2 : Average variation (PM/AM ratio for each treatment S or Sh; Sh/S for each hour AM or PM) in terms of SLW (specific leaf weight) and total sugar concentration of leaf 4 for LT, HT hybrids and Buster and in average across genotypes.

	Leaf 4 SLW variation ($g.cm^{-2}$)			
	hour effect (S)	**hour effect (Sh)**	**Sh effect (AM)**	**Sh effect (PM)**
LT	1.251	1.251	0.818	0.818
HT	1.280	1.381	0.790	0.853
Buster	1.346	1.093	1.017	0.826
Average	***1.29***	***1.24***	***0.88***	***0.83***
	leaf 4 total sugar concentration variation ($mg.g^{-1}$)			
	hour effect (S)	**hour effect (Sh)**	**Sh effect (AM)**	**Sh effect (PM)**
LT	1.250	1.144	1.021	0.935
HT	1.285	1.177	1.008	0.924
Buster	1.244	1.181	0.997	0.946
Average	***1.26***	***1.17***	***1.01***	***0.93***

Model application

a- Calibration

Upgraded version of *EcoMeristem* model was first calibrated on three of the six genotypes investigated by Kim *et al.* (2008b): LT hybrid 1, HT and Buster, studied in the experiment 1 (Exp1) of Kim *et al*. (2008b), i.e. the experiment with the most favourable conditions for tillering. For this purpose, average observations for each of genotype were used to optimize model morphogenetic parameters controlling plant internal competition for C (i.e.,

leaf size and SLA respectively depending on *MGR* and *SLAp* parameters, appearance and expansion rate mainly controlled by *phyllo*, and tillering, mainly affected by *Ict*). Optimization procedures were used to minimize the deviation between simulated and observed variables (target file). Parameter optimization was carried out by generating a large number of simulations by combining ranges of values for the four model parameters considered.

Table 3 presents for each genotype the values of observed variables used to fit model parameters, as well as the resulting optimized parameter values. Figure 5 presents a comparison between simulation outputs (calibrated model) and observations used for calibration.

The model outputs fitted well the corresponding observations, such as shoot dry weight, tiller number and PLA, with very good accuracy for Buster, slightly less well for LT hybrid and least well for HT hybrid (Fig. 5). For LT hybrid, there was a slight delay in the simulated, first tiller emergence but the other model outputs were satisfactory. The HT hybrid (hybrid 4) simulations underestimated both shoot dry weight and plant leaf area for the last date (31 DAG) whereas tiller number was overestimated.

Table 3 : Summary of target data from Exp1 in Kim et al. (2008a) used for model calibration (SDW: shoot dw; Stem number is tiller number including main stem; PLA: plant leaf area; LLLA: last ligulated leaf area in grey in the table) and resulting model parameter values (MGR: meristem growth rate; Ict threshold Ic enabling tillering; SLAp: slope parameter for structural SLA reduction along leaf rank on a given stem; phyllo: phyllochron)

genotype	SDW (g)	stem number	appeared leaf number	ligulated leaf number	PLA (cm^2)	LLLA (cm^2)	MGR (cm)	Ict (unitless)	SLAp	Phyllo (°C.d)
LT	5.59	3.07	10.4	7.4	956.75	143.87	10.08	2.17	65.91	27.1
HT	8.18	3.63	10.8	7.8	1386.6	122.49	8.35	1.50	67.00	26
Buster	8.45	4.53	11.13	8	1338.7	181.3	9.6	1.47	38.64	25

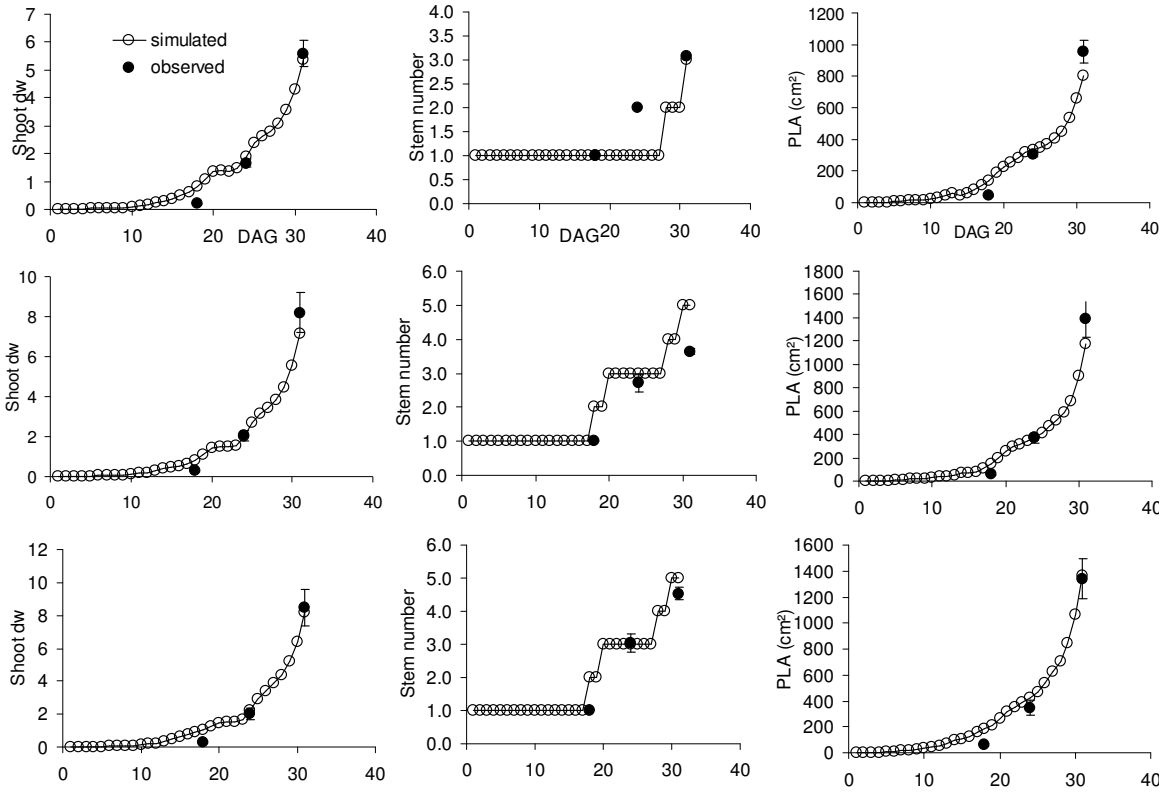


Figure 5 : Comparison between observed (average and standard error) and simulated (calibrated) data for genotypes LT (top), HT (middle) and Buster (bottom) grown in Exp1 (tillering favourable environment). Shoot dw is expressed in g, stem number corresponds to tiller number including main stem, PLA is plant leaf area.

b- Validation

Parameter values resulting from calibration on Exp1 were then used to simulate the behaviour of the same genotypes in an environment less favourable for tillering (Exp3 from Kim *et al.*, 2008b). In Exp1, average daily PTQ was above 2.5 $MJ.m^{-2}$ whereas it was around 1.5 $MJ.m^{-2}$**.** In this environment, the values of *phyllo* used in Exp1 did not simulate phenology correctly. However, by adjusting *phyllo* values (to 34, 36.4 and 35.2 respectively for LT, HT and Buster) while keeping the other morphogenetic parameters identical to those calibrated on Exp1 (see Table 3), simulations of genotype behaviour in Exp3 were good (Figure 6).

In general, simulations accurately predicted observed values for all genotypes, except for an overestimation of earliness of the appearance of the second tiller for HT hybrid.

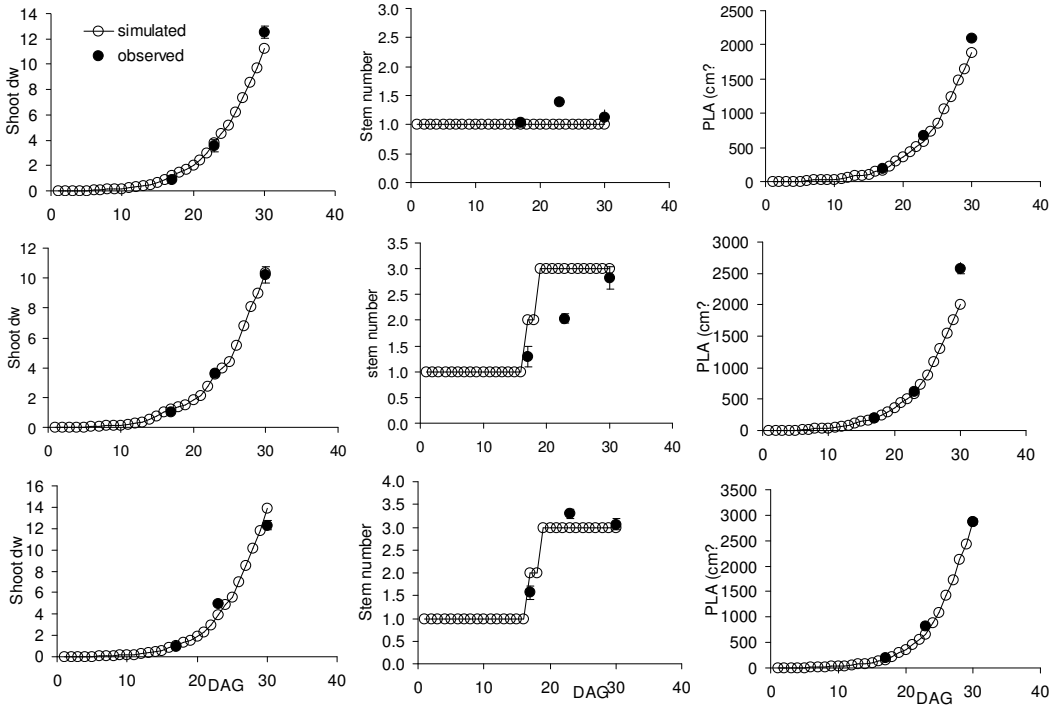


Figure 6 : Comparison between observed (average and standard error) and simulated behaviour of genotypes LT hybrid 3, HT hybrid 4 and Buster in Exp3 from Kim et al. (2008) based on parameter values calibrated on Exp1 (Fig. 5) with an adjusted value of *phyllo* parameter.

c- Sensitivity analysis

A model sensitivity analysis was performed for parameters MGR and Ict, both key parameters controlling tillering as a function of internal competition for C in *EcoMeristem.* Model input variables and other parameters (in particular SLAp, phyllochron) were fixed as average values of the genotypes studied. MGR and Ict were varied between 3-10 and 0.5-2.5, respectively. Sensitivity analysis was carried out using Exp3 meteorological data until the last harvest date (end of tillering phase, leaf 9 ligulated on the main stem). Fig. 7 presents the impact of the variation of MGR and Ict on shoot dry weight (SDW), tiller number, plant leaf area (PLA), C reserve, last fully expanded leaf area (LLLA) and plant SLA. For each combination between MGR in the x-axis and Ict in the y-axis, an output value for the simulated variable give a coordinate in the z-axis. The different colours represent hot spot regions in red, corresponding to optimum combinations, or cold spots in blue.

Results indicated that there is an optimum range of combinations between MGR and Ict values (red peak) for maximal SDW and PLA which is associated with low C reserve status. In fact, according to the model, hypothetical plants expressing this optimum maintain a

sufficiently strong internal demand to convert new assimilates into growth with only minimal transitory storage, but not to the extent that excessive demand would accelerate senescence. The opposite behaviour, associated with maximal of C reserves, corresponds to low SDW and PLA. Tiller number and plant SLA show basically similar trends as observed for SDW and PLA, but there is however a much wider range of MGR and Ict combinations for which tiller number is relatively high and stable, forming a plateau region.

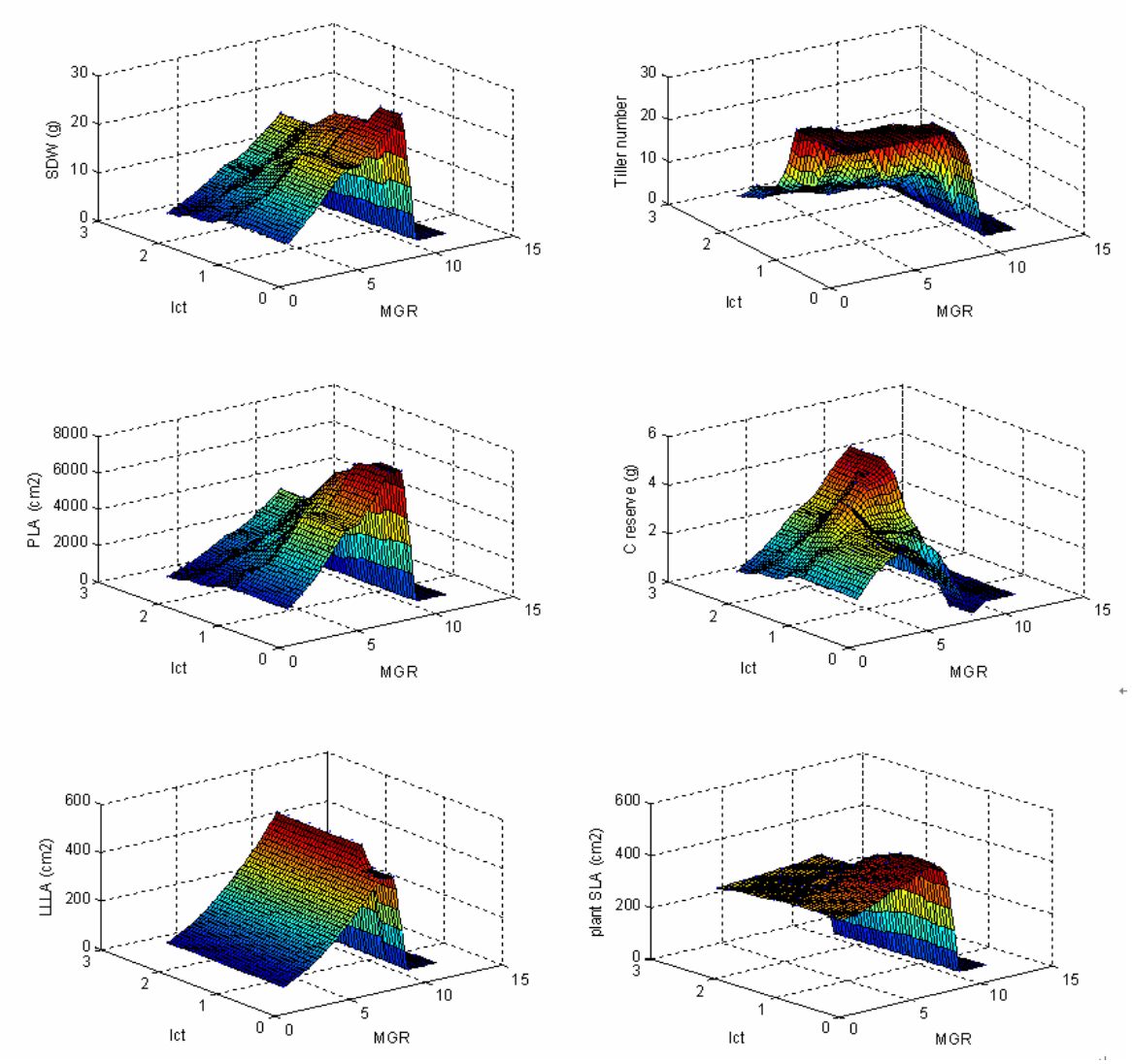


Figure 7 : Impact of MGR and Ict variation on simulated Shoot dry weight (SDW), tiller number, PLA, C reserve, last fully expanded leaf (LLLA) and plant SLA, using Exp3 meteorological data from Kim *et al.* (2008a).

DISCUSSION

A conceptual modelling framework describing the regulation of tillering in sorghum by assimilate availability was developed and tested, providing a basis for improving tillering simulation in existing crop models (Fig. 1, Eq.1, Eq.2). The proposed model puts emphasis on two key aspects to dynamically simulate tillering: (i) the importance of main stem development rate providing sites (buds) for tiller emergence and (ii) the internal competition status for C at the whole-plant level resulting from the sink activity exerted by developing organs, including main stem and tiller borne organs. Environmental effects considered were temperature and radiation regulating main stem phenology as previously shown by Birch *et al.* (1998) and Muchow and Carberry (1990) and leaf development through leaf appearance rate (tip-phyllochron) and leaf ligule appearance rate (lig-phyllochron) as shown by Lafarge *et al.* (1998). Tip- and lig- phyllochrons thereby defined leaf elongation duration (LED) at each successive leaf rank. Thereby, plant assimilate supply and demand for growth at the time of the potential emergence of a new tiller were both largely a function of (main stem) leaf area development. The major environmental effect on plant supply/demand ratio at tillering emergence was captured through its effect on leaf length, characterized through LLIR (Kim *et al*., 2008a), while genotype effects were mainly related to LWIR (Kim *et al*., 2008b). A single equation integrating these environmental and genotypic effects could thus be used to estimate plant internal competition for C and its consequences on tillering dynamics during the vegetative phase according to the framework presented in Fig.1. By analysing tillering through this model, it was shown that the time of emergence of early tiller ranks (T1 and T2) is critical in terms of plant internal competition for C, and most of G and E effects on tillering could be observed during this period.

Some of these concepts were integrated in the existing crop model platform *EcoMeristem*. The optimised model parameters MGR, Ict, SLAp and Phyllo effectively captured the behaviour of LT (hybrid 1), HT (hybrid 4) hybrids and Buster (Tab. 3). Higher MGR (i.e., greater potential increase of leaf size from one phytomer to the next) and higher Ict (i.e. higher C supply/demand ratio required to trigger tiller outgrowth) were found for LT hybrid, and lower MGR and Ict were found for HT hybrid and Buster indeed found. These genotypic characteristics converged with the two principal characteristics shown by trait dissection experiments and formalised through the model equation (Eq. 1): MGR can be related to genotypic difference in leaf size (LWIR) and Ict to the S/D index value above which tillering is observed (Kim *et al.,* 2008b, c).

In the current version of *EcoMeristem* , MGR determines for a given genotype, both potential leaf length and width. In the dicotyledonous model plant *Arabidopsis*, leaf expansion involves at least two independent developmental processes: width development and length development (Tsuge *et al.*, 1996). In wheat, comparison of several *Triticum* and *Aegilops* species species showed that leaf elongation rate (LER) was strongly and positively correlated with leaf width but not with leaf elongation duration (LED) (Bultynck *et al.*, 2004).

The study of Kim *et al.* (2008c, Chapter IV), aiming at dissecting the tillering process into component traits such as LWIR and relating them to QTLs, indicated that genotypic differences in tillering ability is negatively correlated with leaf width. Differential tillering ability was thereby interpreted as being a result of differential levels of competition for C: the production of larger organs involves greater demand for C and thus limits tillering. In rice, dwarf and semi-dwarf cultivars, which generally have reduced leaf size, show high tillering ability, and some genomic studies indicate a direct, positive link between dwarfing and tillering (Ishikawa *et al.*, 2005).

It is unclear why tillering ability was more related to genotypic differences in leaf width than to leaf length or area (Kim *et al.*, 2008c). Reymond *et al.* (2004) showed that leaf width is a constitutive trait in maize. It is thus possible that leaf width is more strongly genetically determined, whereas final leaf length is influenced by environment throughout the expansion process. If this hypothesis is true, the MGR parameter of *EcoMeristem* should control mainly potential leaf width, whereas no potential value should be simulated for final leaf length because the elongation processes is highly environment dependent.

Regarding the performance of the *EcoMeristem* model, a major concern was the lack of stability across environments of the Phyllo parameter, which sets the development rate. The model simulates a constant phyllochron in terms of thermal time, except under conditions of acute assimilate shortage, a condition which slows down organ expansion rates and as a consequence, organ appearance rates. The variation of observed phyllochrons in Exp 1 and Exp 3 could not be explained by the model, and it was therefore necessary to force the Phyllo parameter for Exp 3 (validation study). No such stability problems were encountered with parameters MGR and Ict. This experience confirms the fact that simulating plant phenology accurately across environments is a pre-requisite to the implementation of tillering models (Bos and Neuteboom, 1998).

The slight divergence between observed and simulated values of shoot dry weight while leaf area is well simulated, in particular for the HT hybrid, can be explained with an excessively high allocation of C to the reserve compartment (explaining the disequilibrium observed between PLA and dw simulation in Fig.5 and Fig.6). This might be further

explained by the fact that tillering simulation in *EcoMeristem* is binary and only considers integer values (at average plant level), whereas in observed data, decimal values could be found for tiller number (as they were means of ten plants). Finally, the probable ability of high tillering hybrids to produce thinner leaves (higher SLA) was not well captured by the model. However this latter hypothesis could not be statistically confirmed by field data as the error of leaf area and dry weight measurements was too large. In rice, dwarf habit and high tillering rates are frequently associated with high SLA (Dingkuhn *et al.*, 2001).

The complementary experiment reported in this paper exploring G and E effects on plant carbohydrate concentration at pre-tillering stage was undertaken to support the modelling concepts described above. A marked, transitory accumulation of reserves was observed from morning (AM) to afternoon (PM), particularly for starch in leaf blades. It was demonstrated that this transitory pool explained much of the diurnal variation of SLA. Genotype effects on this variation, however, were small or non-existent. Shade treatment reduced carbohydrate accumulation in the afternoon, but slightly increase remaining reserves in the morning, resulting in a reduced amplitude of diurnal reserve oscillations. This observation is in accordance with the reduced photosynthetic activity that can be expected under shade. Shade also reduced structural leaf weight per unit area, a well-known phenomenon known as sun or shade leaf morphology.

Although the experimental results on carbohydrate dynamics could not be directly and quantitatively compared with Exp1 or Exp3 field results (Kim *et al.*, 2008a,b) and with *EcoMeristem* simulation outputs because neither included data on carbohydrate concentration, some interesting trends were observed across these studies. It was observed that the highest-tillering genotype Buster had the lowest leaf starch concentration in the morning, possibly indicating that translocation during the night was efficient due to strong demand (sinks) within the plant (Table 4). Under shade, these genotypic differences were conserved but at a higher level of reserves in the morning, possibly indicating that sink attraction for assimilates was smaller. It thus seems that genotypic tillering ability was inversely related to plant carbohydrate status. The results are only preliminary, however, and more experiments are required to confirm the present interpretation.

Table 4: Comparison of genotype characteristics across experimental studies: Leaf blade starch content in the morning (AM; from Fig. 3) for Sun and Shade treatments, total tiller number per plant in Exp1 (Kim *et al.*, 2008a) and simulated shoot reserves at pre-tillering and tillering stages for Exp1.

Genotype	Leaf starch (AM), $mg.g^{-1}$ (Exp described in this paper)		Tillers.$plant^{-1}$ (observed, Exp1)	Tillers.$plant^{-1}$ (observed, Exp3)
	Sun	Shade		
LT	11.0 ± 0.6	22.3 ± 1.8	2.0	0.7
HT	12.7 ± 2.7	18.9 ± 1.6	2.6	1.7
Buster	6.6 ± 0.4	8.0 (*)	3.5	2.5

(*) missing reps

This behaviour can be simulated with *EcoMeristem*, as demonstrated in the combined sensitivity analysis for MGR and Ict parameters (Fig. 7). But depending on parameterization, simulated tillering ability can be positively or negatively correlated with reserve status, the former if MGR parameter is varied and the latter if Ict parameter is varied (Fig. 8). The explanation is that if tillering is increased by lowering the genotypic Ict threshold, demand for assimilates increases in the plant and transitory reserves decrease. On the other hand, if tillering is increased through weaker competition for assimilates, which is the case when a lower genotypic MGR causes the production of smaller organs, reserve levels in the plant increase.

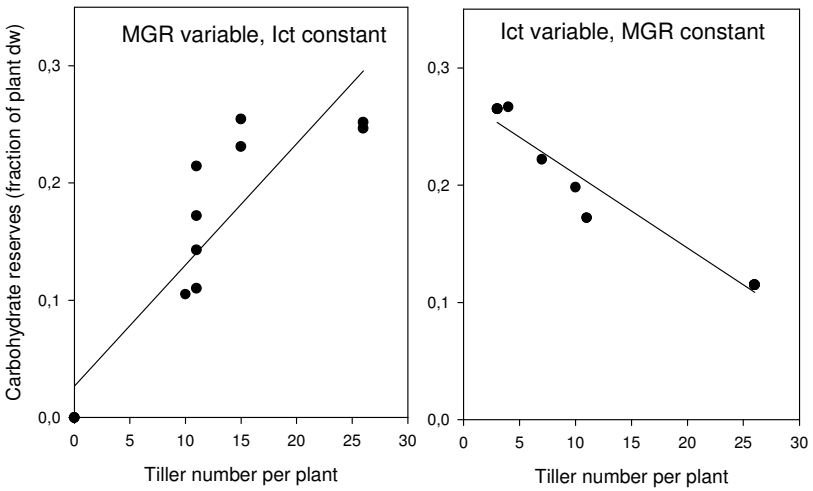


Fig. 8. Sensitivity analysis of *EcoMeristem* model using Exp1 (Kim *et al.*, 2008a) environment: Left, relationship between simulated plant C reserves and tiller number for variable levels of MGR parameter. Right, the same analysis for variable levels of Ict parameter.

Kim *et al.* (2008a,b,c) found negative correlations between leaf size parameters and tillering ability across genotypes. This observation was interpreted in terms of constitutive genotypic differences in leaf size that indirectly affect tillering through competition among sinks for assimilates, particularly during the pre-tillering phase of development. This would translate, in terms of the *EcoMeristem* model concept, into genotypic variation of the MGR parameter, resulting in a positive correlation between reserve status and tiller number across genotypes (Fig. 8, left graph). On the other hand, the preliminary results reported here on carbohydrate status during pre-tillering seem to suggest the opposite (Table 4): genotypic tillering ability was (loosely) associated with more exhaustive leaf reserve mobilization during the night, suggesting that tillering ability was constitutive (genotypic variation in Ict, Fig. 8 right graph).

In fact, parameter optimization for the 3 genotypes studied resulted in genotypic differences in both MGR and Ict (Table 3), high tillering Buster having the smallest MGR (producing comparatively small leaves, which should theoretically increase tillering) and the smallest Ict (enabling tiller initiation at comparatively low assimilate availability). Simulations applying these parameters to Exp1 gave virtually identical transitory reserve levels (daily means) for all 3 genotypes (data not presented). This observation might indicate that among the infinite number of genotypic parameter combinations that can be generated and tested with the model (example: 3D surfaces in Fig. 7), only certain and possibly narrow

ranges of combinations exist in reality. Others are either biologically impossible or have been deselected by nature and breeding.

Such *in silico* experiments by means of sensitivity analysis are particularly useful to explore virtual genotype behaviour by combining various combinations of component traits, corresponding to different parameter values. The present analysis showed that complex traits such as tillering, which are subject to strong GxE interactions because they are resource dependent, can be an expression of different genetic and physiological, interacting factors. Combinations of component traits achieving the same result in terms of tiller number may incur different tradeoffs in terms of plant leaf area, biomass or water use, and eventually yield. Further model improvement and validation efforts are needed, however, before a complex tool such as *EcoMeristem* can reliably serve such purposes.

The model might also be of value for further research on environmental effects of complex trat expression such as tillering and other yield components. Internal resource levels in the plant, as simulated by *EcoMeristem*, can affect growth directly through deficiency, but also through signalling on the organogenetic and morphogenetic process, here simplified in the form of a summary supply/demand ratio (Ic in the model). Different genotypic Ict thresgholds for tillering thereby stand for different physiological sensitivity to the Ic (assimilate availability) signal. The three genotypes studied may thus have different sensitivity to sugar signals (Roitsch *et al.*, 2000; Rolland *et al.*, 2006; Wingler *et al.*, 2006). Indeed, sugar plays a key role as a signal as well as a substrate. This is in particular the case of sucrose (Liu *et al.*, 2004; Vaughn *et al.*, 2002; Yang *et al.*, 2003), playing a key role in sugar transport within the plant so that the capacity of sources to produce sucrose matches the capacity of sinks to consume it (Farrar *et al.*, 2000). This might be also explain the observation that the three genotypes showed different response to Sh regarding morphogenetic components (SLAstruc and LA in Figure 4). Modelling approaches are thus potentially useful to evaluate such genotype dependent sensitivity to sugar availability.

CONCLUSION

This study aimed at linking a conceptual model of sorghum tillering as affected by competition for assimilates among sinks, tested previously on several environments and genotypes (Kim et al., 2008abc), to the *EcoMeristem* model simulating phenotypic plasticity (Luquet et al., 2006). It thereby provided new data on diurnal and radiation dependent changes of carbohydrate distribution in the plant at pre-tillering in three contrasting cultivars, and sought to relate them to their known tillering potential through modelling.

The marked accumulation CH_2O in the shoot in the afternoon did not differ among genotypes but was reduced by shading. It contributed to diunal dynamics of SLA but not to shade effects on SLA. Its largest component, starch concentration in the leaf, however, was smallest in the morning for the highest tillering genotype, Buster. Net starch accumulation during the day in the leaf thus showed a positive relationship with tillering potential but this trend needs further confirmation.

EcoMeristem sensitivity analysis showed that according to the model, high genotypic tillering potential can be either positively or negatively correlated with plant CH_2O reserve status, depending on whether direct constitutive tillering response to CH_2O (parameter Ict) is at work, or alternatively the level of competition among other sinks, here related to leaf size (parameter MGR). Evidence produced by Kim et al. (2008abc) tends to suggest the latter (leaf size effects), but the present study provides no decisive clues for this hypothesis. Parameterization of *EcoMeristem* on the three genotypesis suggested that both MGR and Ict varied among the cultivars, which would indicate that both leaf size and tillering ability are somewhat directly constitutive, but interact in determining the resulting phenotype.

It is concluded that the present approach of combining heuristics (model based analysis) and physiological experiments can contribute to the understanding of complex traits such as tillering, and by extension, can serve as a basis to study virtual plants combining different component traits constituting the complex traits. To do this with sufficient confidence, however, the hypotheses and models have to mature further.

"There is a time for everything,
and a season for every activity under heaven:
a time to be born and a time to die,
a time to plant and a time to uproot,
a time to kill and a time to heal,
a time to tear down and a time to build,
a time to weep and a time to laugh,
a time to mourn and a time to dance,
a time to scatter stones and a time to gather them,
a time to embrace and a time to refrain,
a time to search and a time to give up,
a time to keep and a time to throw away,
a time to tear and a time to mend,
a time to be silent and a time to speak,
a time to love and a time to hate,
a time for war and a time for peace…

I know that there is nothing better for men than to be happy and do good while they live.
That everyone may eat and drink, and find satisfaction in all his toil…"

CHAPTER VI

SUMMARY, PERSPECTIVES & CONCLUDING REMARKS

1. SUMMARY

Initial hypothesis

The objective of this PhD study was to unravel the genotypic and environmental control of sorghum tillering regulation by plant internal competition for C. Tillering was chosen as a highly plastic (G and E dependent) key component trait of yield in cereals. Sorghum (*Sorghum bicolor* (L.) Moench) is a crop of high agronomic interest especially in water limiting cropping environments (e.g. in Western Africa and Australia) and in terms of genetics, its full genome sequence is now available (Bowers *et al.*, 2007). Thus it was chosen as a model crop in this study to carry out a morphogenetic and ecophysiological 'dissection' of tillering response to C availability.

The key hypotheses of this work were:

- during the early vegetative phase, initiation of tiller bud outgrowth is controlled by C availability whereas its cessation is associated with sensitivity to red:far-red ratio, (Lafarge and Hammer, 2002; Evers *et al.*, 2006)
- C availability for tiller bud outgrowth would depend both on E, in particular photothermal conditions, (Bos and Neuteboom, 1998; Honda and Okajima, 1970) and G, in particular morphogenesis effects on plant internal competition for C (Dusserre *et al.*, 2002; Luquet *et al.*, 2006)
- by dissecting tillering (complex trait) response to C availability into component traits, it becomes possible to separate and formalize its G and E determinisms in a modelling framework allowing its simulation as an emergent property of dynamic processes (Cooper *et al.*, 2005; Dingkuhn *et al.*, 2005; Hammer *et al.*, 2005; Yin *et al.*, 2004)
- the parameters of such a modelling framework would be more powerful in dissecting the genetic bases of tillering (QTL detection) than tiller number alone as its phenotyping would be prone to GE interactions (Baenziger *et al.*, 2004; Hammer *et al.*, 2002)

- such knowledge on tillering could add value to existing crop models that are not capable of generating the G and E effects influencing yield through their impact on tillering (Hammer *et al.*, 2006; Yin *et al.*, 2003)

- **E and G regulation of sorghum tillering by plant internal competition for C**

In this study, tillering regulation by C availability was shown to be strongly dependent on main stem development and leaf morphogenesis:

1. There was a consistent coordination (across environments and genotypes) between the appearance of a given tiller rank and of a given leaf rank on the main stem, resulting in an inherent competition for resources between expanding leaves and tiller outgrowth. This supported results presented by other authors on sorghum or other cereals ((Bos and Neuteboom, 1998; Jaffuel and Dauzat, 2005; Lafarge *et al.*, 2002).
2. The main E effect was observed on the appearance frequency of the lower-rank tillers similar to the density effect shown by Lafarge *et al.* (Bos and Neuteboom, 1998; Lafarge *et al.*, 2002)
3. Photo-thermal quotient (PTQ), which indexed growth per unit development, was the key E variable to be considered to explain tillering regulation by plant internal competition for C, because of its direct role on C assimilation (via radiation) and its influence on leaf area development (e.g. leaf length, tillering phase duration etc.) (via temperature). This provided further insight into how PTQ was previously considered in other studies (Nix, 1976; Ortiz-Monasterio *et al.*, 1994)
4. The main G differences were observed on tiller onset frequency and thus on maximal tiller number, which was negatively related to main stem LWIR as suggested for rice by Tivet *et al.* (Tivet *et al.*, 2001)

Those results were consistent with the hypothesis that plant internal competition for C regulates tillering in sorghum, as already suggested for high tillering cereals such as wheat

(Friend, 1965), rice (Honda and Okajima, 1970), barley (Kirby and Faris, 1972), and ryegrass (Ong and Marshall, 1979). However in these previous studies, neither proof nor quantification of underlying GE processes was provided. Here, a detailed analysis of G and E determinisms of such processes enabled elucidation of a modelling framework relying on (i) the coordination between main stem morphogenesis and tillering and (ii) a simple equation defining the C supply/demand status at a given plant (main stem) phenological stage.

- **Generic modelling framework**

The S/D_{index} elaborated in this PhD study aims at estimating, in a simple and robust way, the competition for C existing within the dynamic framework of coordination between main stem morphogenesis and tillering. In this index, the G or GE control of leaf area development plays a major role in the estimation of both C supply and demand. This is in agreement with the way C supply and demand are already conceptualized at a broad level in some existing models such as Greenlab (Yan *et al.*, 2004), *EcoMeristem* (Luquet *et al.*, 2006), APSIM (Wang et al., EJA paper), and GRAAL (Drouet and Pagès, 2003) that dynamically simulate whole plant phenotype. However, the key advance of the S/D_{index} defined here lies in the identification of experimentally derived indicators of C supply and demand that explain most of the variability in tillering across the range of E and G studied:

- G variability encountered in terms of leaf demand for C on a given stem was largely captured through differences in LWIR during the tillering phase. The potential impact of G variation in phyllochron (i.e. leaf developmental rate) on plant internal competition for C was not observed in this study, even though it has already been shown to impact tillering in previous studies (Borrell *et al.*, 2000; van Oosterom *et al.*, 2008).
- The area of leaf 5 was shown as a robust indicator of plant leaf area (and thus of C supply acquisition ability) variability across G and E at the tillering onset stage.

The key role of leaf area development in determining both C supply and demand in S/D_{index}

strongly supports the importance of correctly simulating leaf area dynamics in crop models, which in turn reinforces the importance of accounting for tillering (Lafarge and Hammer, 2002). This becomes even more crucial when crop models are aimed at dealing with water use efficiency issues depending on E and G combinations (Hammer, 1998; Hammer, 2006; McLean *et al.*, 2003; Muchow *et al.*, 1994).

Based on S/D_{index}, it is suggested that a surplus of C allows an axillary bud to grow out as a tiller, provided this occurs within a window of potential appearance in relation to environmental conditions and leaf appearance (Cline, 1994; Dun *et al.*, 2006; Shimizu-Sato and Mori, 2001; Tomlinson and O'Connor, 2004). This is similar with what was proposed by Luquet *et al.* (2006) for rice.

Similarly to what was proposed for onset of tillering, it was suggested in Chapter II and III that tiller fertility or senescence could be also explained by internal competition for C availability at the whole plant level. This confirms the modelling framework for tiller fertility proposed by Lafarge and Hammer (2002). This is an important result regarding the objectives of extending the *EcoMeristem* model to the reproductive phase and of implementing GE process control of sorghum tillering in APSIM.

Analyses of carbohydrate concentration among the different growing organs suggested that genotypic tillering ability was inversely related to plant carbohydrate status. Low tillering genotypes retained greater levels of starch in leaves. However, these preliminary results require confirmation.

- **Complex trait ecophysiological dissection and genetic studies**

The dissection of tillering control by C availability into component (process based) traits was intended to narrow the gap between a complex phenotypic trait and the underlying genetic control. This was achieved in this study as the key genotype dependent component traits identified were able to link to putative QTL for tillering across eight BC2F2 populations

(mainly LWIR, S/D_{index} threshold related to QTLs on LG4 and LG3 respectively). Traits such as leaf width were already shown to have higher heritability (Tuberosa *et al.*, 2002) than LAI, SLA (Yin *et al.*, 1999) or other integrative traits. A major difficulty in the use of QTLs of complex traits is indeed their instability depending on E (e.g. maize QTLs related to flowering dates (Ribaut *et al.*, 1996), QTLs of leaf abscisic acid (Tuberosa *et al.*, 1998); wheat QTLs related to lodging, (Keller *et al.*, 1999)). There are a number of reports on association of QTLs to leaf width in wheat (Quarrie *et al.*, 2006; Rebetzke *et al.*, 2004) or maize (Reymond *et al.*, 2004), and specific genes controlling leaf width have been identified in *Arabidopsis* (Tsuge *et al.*, 1996; Tsukaya, 2005). Such a morphogenetic trait is assumed to be less prone to GE interactions that complicate breeding procedures and limit the usefulness of selection in only one environment (Baker, 1988; Easton and Clement, 1973).

As mentioned above, a genotypic S/D_{index} threshold for tillering was suggested by sugar analyses in sorghum plants and confirmed both by *EcoMeristem* model application and by the association of such a threshold with a putative QTL for tillering. This is a key result of this PhD work. Indeed, Luquet *et al.* (2006; 2008) already showed for rice the existence of such a genotype dependent sensitivity of tillering to C availability (Ict parameter). However, the relation of such a process based parameter with genetic information has not been found previously.

Co-localization of such QTLs (LWIR, S/Dindex threshold) with QTLs related to stay-green trait and yield in other populations within the DPI breeding program germplasm (Jordan, personal communication) is promising and also demonstrates the added value of collaboration between physiologists, modellers, geneticists and breeders.

In parallel, the fact that some of the BC2F2 populations studied for model assisted QTL analysis did not show any relationship between tillering and LWIR indicates that in the present study only one of several regulation pathways of tillering was addressed. We also investigated a candidate gene approach by targeting maize *tb1* gene (Clark *et al.*, 2004;

Hubbard *et al.*, 2002) homologue in sorghum by developing three specific primers (results not shown). Two of them showed different alleles between *Sorghum arundineceum.* (wild type parent) and the recurrent parent (R931945-2-2) but the BC1F4 parental lines we selected had all fixed the recurrent parent alleles. Thus in our case, the *tb1* gene was not involved in the tillering regulation mechanism we investigated. However it might be worth extending the approach to other candidate genes. In the same context, significant progress has been made in isolating and characterizing genes in *Arabidopsis* that are directly involved in the formation of plant architecture, especially those controlling the initiation and outgrowth of axillary buds, elongation of stems and architecture of inflorescences. Most of these genes are conserved between dicotyledonous and monocotyledonous plants, indicating that these plants share similar regulatory pathways to build their body plant (Wang and Li, 2006). The conservation of these genes makes them of great agronomical importance for improving crop yield in the future.

- **Role and added value of modelling in complex trait studies and G to P analyses**

The adaptation of *EcoMeristem* (Luquet *et al.*, 2006) to sorghum based on some of the concepts elaborated in this study enabled use of this model to simulate tillering of three contrasting sorghum hybrids in two contrasting photo-thermal environments. *Ecomeristem* parameter values optimized for each of those genotypes confirmed what was observed at the level of genotypic components of S/D_{index}: MGR (*Ecomeristem*) parameter controlling leaf size (thus related to LWIR) was higher for low tillering, big leaf genotypes and associated with a high Ict parameter, controlling in *Ecomeristem* the threshold of S/D enabling tillering (thus related to S/D_{index} threshold). As S/D_{index} genotypic coefficients were closely associated with identified QTLs for tillering (Chapter IV), such a QTL effect could be introduced into the model to scale genetic information up to consequences at the crop level. The relevance of this integrative approach has already been demonstrated by Chenu *et al.* (2008), who

incorporated QTLs related to leaf elongation rate of maize into the APSIM crop model to simulate their impact on LAI and yield. By introducing genetic information into crop models, it becomes possible to explore some QTL combinations and quantify adaptation landscapes in a manner relevant to plant breeding (Hammer *et al.*, 2006). On the other hand, crop modelling can also be a powerful tool to unravel GE and to dissect complex traits into characters that might be under simpler genetic control and thus useful for phenotyping (Dingkuhn *et al.*, 2006).

PERSPECTIVES

Connecting physiological and genetic information

- Preliminary results on possible physiological mechanisms mediated by sugar concentration pointed out that genotypic tillering ability was inversely related to plant carbohydrate status (mainly starch in leaves and sucrose in sheath compartment) but other experiments (with precise control of radiation and temperature) are required as well as extending the analyses to other phases, i.e [TEM] and [TSEN].
- The genetic analysis was conducted on a limited number of genetic markers (SSR) but other segregating regions, in particular on LG1 and LG6, are expected to be genotyped in the different populations studied, providing new opportunities for QTL detection based on component traits of tillering.
- Experimental studies in other environments are needed to validate detected QTL stability
- Availability of the sorghum genome sequence provides opportunity for comparative genomics and candidate gene approach based on homologue genes identified in other species regarding branching (e.g. *Arabidopsis thaliana*, *Oryza sativa*)
- This study generally provides a robust framework to apply similar trait dissection methods to the study of other complex traits study

Integrating physiological knowledge into models

- Implementation of developed modelling concepts into two models are underway
    - In *EcoMeristem,* further adaptations are under consideration for sorghum such as the introduction of a time window of opportunity for tiller outgrowth depending on their rank and the use of developed concepts to support model extension to reproductive phase (tiller fertility and influence on yield).
    - In APSIM, incorporation of developed tillering modelling concepts is currently underway as a module accounting for GE regulation of tillering and thus enabling a more process-based simulation of plant leaf area development. This will give better insight into the analysis of effects of GME interactions on grain yield, in particular in water limited environments (McLean *et al.*, 2003).
    - As S/D_{index} genotypic coefficients could be connected to QTLs, it offers the opportunity to introduce QTL effects into crop models and thus further test G, GE or GME effects on tillering, leaf area dynamics and yield.
- Upgraded models to assist genetic analysis
    - Combining genetic information to genetic parameters based on a new 'generation' of models might open new opportunities for supporting breeding, by exploring '*in silico*' virtual genotypes combining different alleles of QTL effects and supporting the design of ideotypes adapted to specific environmental conditions.
    - In the case of tillering, it might help in selecting the behaviour specifically adapted to prevalent management*environment systems in a target region and acceptable for farmers at a socio-economical level (Mekbib, 2006).

CONCLUDING REMARKS

This PhD study aimed at unravelling the G and E determinisms of tillering regulation by plant internal competition for C in the case of sorghum. The key results of this study are:

- Tillering dynamics is highly coordinated with main stem leaf morphogenesis that results in an inherent competition for C within the plant for tiller outgrowth; such coordination is consistent across environments and genotypes showing that competition for C affects primarily the frequency of low rank tiller appearance;
- Based on these experimental results, an indicator of plant internal competition for C (S/D_{index}) was formalised and explained most of the variation in tillering observed across five environments and six genotypes. S/D_{index} considers, based on easily accessible phenotypic measurements, the G and E determinants of plant leaf area development involved both in plant C supply and demand.;
- Genotypic differences in the relationship between the S/D_{index} and tillering rate suggested high-tillering hybrids have a lower S/D threshold at which tillers appear.
- E and G determinisms of C availability regulation of tillering were in part confirmed by an analysis of sugar distribution and concentration in sorghum plants

By using genotypic components of S/D_{index} in a genetic study, it was possible to:

- Relate directly 2 of 3 putative QTLs associated with tillering to its component traits (LWIR or S/D_{index})
- Illustrate how complex trait modelling (i.e. dissection into elementary process) can assist genetic studies in a manner relevant to breeding programs, assuming component traits are more genetically heritable and related to a smaller number of genes.

First attempts to upgrade *Ecomeristem* model with the elaborated concepts showed that:

- Adapted *EcoMeristem* version to sorghum was able to simulate tillering across contrasting genotypes and environments

OPENING THOUGHTS (to Chapter I)

Luke 12:27-29

TRANSITION THOUGHTS (to Chapter II)

Matthew 13:3-8

TRANSITION THOUGHTS (to Chapter III)

Mark 4:30-32

TRANSITION THOUGHTS (to Chapter IV)

Psalm 103:15-16

Job 14:7-9

TRANSITION THOUGHTS (to Chapter V)

Deuteronomy 32:2

Psalm 102:4

Matthew 16:2-3

CLOSING THOUGHTS (to Chapter VI)

Ecclesiastes 3:1-8;12-13

RE-OPENING THOUGHTS (to APPENDICES)

Psalm 23:1-6

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CHAPTER I

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"Il y a un moment pour tout,
un temps pour toute chose sous le ciel:
Un temps pour enfanter et un temps pour mourir ;
Un temps pour planter et un temps pour arracher le plant ;
Un temps pour tuer et un temps pour guérir ;
Un temps pour démolir et un temps pour bâtir ;
Un temps pour pleurer et un temps pour rire ;
Un temps pour se lamenter et un temps pour danser ;
Un temps pour jeter des pierres et un temps pour ramasser des pierres ;
Un temps pour étreindre et un temps pour s'éloigner de l'étreinte ;
Un temps pour chercher et un temps pour perdre ;
Un temps pour garder et un temps pour jeter ;
Un temps pour déchirer et un temps pour recoudre ;
Un temps pour se taire et un temps pour parler ;
Un temps pour aimer et un temps pour haïr ;
Un temps de guerre et un temps de paix.
…
Que reste-t-il à celui qui travaille de la peine qu'il prend ?
…
J'ai reconnu qu'il n'y a rien de bon pour lui
sinon de se réjouir et de faire ce qui est bon pendant sa vie ;
et aussi que pour tout homme,
manger boire et voir ce qui est bon au milieu de son travail,
est un don de Dieu. "

RESUME EN FRANÇAIS

Chapitre I

Introduction

L'objectif de cette thèse était la compréhension du contrôle environnemental (E) et génétique (G) de la régulation du tallage par la compétition interne à la plante pour les assimilats carbonés chez le sorgho. Le tallage a été choisi en tant que composante morphogénétique clé et extrêmement plastique du rendement des céréales, dépendant de facteurs E et G. Le sorgho (*Sorghum bicolor* (L.) Moench), plante cultivée de grand intérêt agronomique en particulier dans les environnements limitant en eau (e.g. Afrique sub-saharienne, Australie), et par ailleurs deuxième céréale après le riz dont le génome a été complètement séquencé (Bowers *et al.*, 2007), a été choisi comme plante modèle pour les monocotylédones C4, d'autant plus approprié que son tallage modéré facilite son analyse fonctionnelle et génétique de son tallage.

Les hypothèses d'étude explorées au cours de ces travaux de thèse on été :

- pendant la phase végétative précoce, l'initiation de la croissance des bourgeons axillaires (talles) est contrôlée par la disponibilité en C (Lafarge and Hammer, 2002) alors que leur cessation, lorsque la compétition pour la lumière est plus forte, est sous le contrôle de la photosensibilité au rapport rouge clair : rouge sombre (Evers *et al.*, 2006)
- la disponibilité en C pour l'émergence et la croissance des talles dépend à la fois de facteurs E, en particulier caractérisés par les conditions photo-thermiques, et de facteurs G, notamment les caractéristiques morphogénétiques des feuilles qui définissent le niveau de compétition en C dans la plante
- en décomposant la dynamique du tallage en caractères élémentaires, il serait possible de formaliser de façon distincte dans un modèle les déterminismes E et G, et ainsi

permettre de simuler de façon robuste au travers des génotypes et environnements le tallage comme un processus dynamique consécutif à la disponibilité en assimilats C (Cooper *et al.*, 2005; Dingkuhn *et al.*, 2005; Hammer *et al.*, 2005; Yin *et al*., 2004)

- les paramètres d'un tel modèle peuvent ensuite être utilisés en tant que caractères phénotypiques plus proches des bases génétiques du tallage (pour la détection de QTLs) faisant du modèle un outil d'appui au phénotypage, permettant de s'affranchir des effets GxE (Baenzinger *et al*., 2004; Hammer *et al*., 2002)
- l'intégration de telles connaissances dans des modèles de croissance du peuplement tels que *EcoMeristem* ou APSIM permettraient donc de mieux simuler le tallage, son impact sur la croissance, le rendement et la gestion de l'eau par la culture (Hammer *et al*., 2006; Yin *et al*., 2003)

Cette thèse est organisée en six chapitres distincts : une introduction générale (I) suivie de quatre chapitres sous formes d'articles scientifiques (II-V) et une discussion générale avec une synthèse des principaux résultats (VI).

L'introduction (chapitre I) présente l'état de l'art concernant l'importance du tallage chez les céréales dans un contexte général mais en mettant l'accent sur les avantages de l'étude chez le sorgho en tant que plante modèle du point de vue génétique et physiologique. Le rôle possible de la modélisation dans le cadre d'études physiologique et génétique de caractères complexes est aussi présenté dans ce premier chapitre.

Les résultats décrits dans les chapitre II et III tentent de démontrer que la régulation du tallage est étroitement liée à sa compétition avec le développement et la croissance des différents axes existant (en particulier la morphogenèse des feuilles du brin maître au cours de la phase de tallage), affectant notamment la fréquence d'apparition des talles précoces. Un indicateur de compétition en C dans la plante, S/D_{index}, permettant d'expliquer le tallage au travers de la gamme étudiée de G et E, a ainsi été développé.

Le formalisme de modèle ainsi élaboré expérimentalement a été appliqué pour appuyer une étude génétique (chapitre IV) en vue d'identifier des QTLs associés au tallage et notamment aux paramètres du modèle relatifs à la largeur des feuilles et à un seuil de tallage. L'ensemble de cette approche de modélisation a aussi été confortée par une analyse de la distribution des sucres dans la plante et a permis d'initier l'amélioration et les tests d'un modèle existant (*EcoMeristem*, chapitre V).

Cette thèse développe ainsi un cadre conceptuel de modélisation formalisant les composantes G et E du contrôle du tallage chez le sorgho (plante modèle), fondé sur l'hypothèse que la disponibilité en assimilats carbonés (C) (estimée par le rapport offre/demande, S/D) est un facteur déterminant. Cette approche a été élaborée et testé au travers de six expérimentations (au champ et en milieu contrôlé) et six génotypes aux capacités de tallage différentes.

Chapitre II

Régulation du tallage chez le sorgho : Effets environmementaux

Introduction

Le tallage peut contribuer dans certaines configurations agro-environnementales de façon significative au dévelopement du couvert végétal (de la canopée) en favorisant l'interception de l'énergie solaire, la vigueur de croissance de la culture puis le rendement du sorgho. Cependant, les bases écophysiologiques du tallage et notamment sa régulation environmentale et génétique ont été très peu abordées, en tout cas pas assez pour fournir des bases à son intégration dans une démarche de sélection variétale. L'objectif de cette première partie de l'étude est de comprendre et quantifier les effets environmentaux sur le tallage chez le sorgho.

Matériel & méthodes

Une série de cinq expérimentations, dont trois au champ et deux en milieu controlé, a généré une large gamme de conditions photo-thermiques. Dans un premier temps, la dynamique de tallage d'une variété représentative a été suivie au cours de la phase végétative (jusqu'à l'anthèse). Le concept de compétition interne pour les carbohydrates a été testé par l'intermédiaire d'une équation simple (S/D_{index}) intégrant les principales composantes de l'offre et de la demande en C au niveau de la plante.

Résultats

L'apparition des talles est un processus fortement coordonné avec l'apparition des feuilles sur le brin maître et présente une hiérarchie stable de fréquence apparition au travers des environments. Le principal effet environnemental réside en effet dans la fréquence de tallage, en particulier sur les rangs de talles précoces (T1 et T2). Cet impact sur les talles T1 et T2 s'explique par la variabilité des conditions environnementales décisivé en début de phase de tallage. Un indicateur générique de l'état de compétition interne à la plante pour les assimilats carbonés (S/D_{index}), relativement simple et accessible par l'expérimentation, a été élaboré et a permis de quantifier, expliquer la variation du nombre maximum de talles observé au travers des cinq expérimentations.

Conclusions

Les effets environmentaux (photo-thermiques) sur le tallage sont en accord avec l'hypothèse de régulation du tallage comme conséquence de la compétition interne de la plante pour les assimilats carbonés. Par conséquent, leur formalisation dans le cadre d'un modèle peut permettre d'améliort sa valeur prédictive en termes de dynamique du tallage et donc du couvert, de l'utilisation de l'eau et des ressources et du rendement.

Chapitre III

Régulation du tallage chez le sorgho : Effets génotypiques

Introduction

Le tallage est un des caractères le plus plastique de la phase végétative affectant notamment la biomasse (vigueur végétative) et le rendement pour les cultures cultivées. Sa variation génétique affecte par conséquent la dynamique du couvert végétale, l'utilisation des ressources en eau et donc le moment et la nature du stress hydrique. Faisant suite aux résultats du chapitre I, l'objectif de cette seconde partie de l'étude est de développer un formalisme intégrant le contrôle génotypique et environnemental (et leurs interactions) du tallage comme une conséquence de la compétition interne pour les assimilats carbonés au niveau de la plante.

Matériel & méthodes

Cinq hybrides, issus de lignées recombinantes sélectionnées pour une phénologie et une hauteur de plante similaires mais une taille de feuilles et une capacité de tallage suffisamment contrastées, ont été semés dans cinq environnements ayant généré une large gamme de conditions photothermiques et de compétition pour les assimilats dans la plante. La dynamique de la surface foliaire de la plante, l'accumulation et la répartition de la biomasse ont été caractérisées à intervalles réguliers. L'état de compétition interne pour les assimilats carbonés a été estimé avec le S/D_{index} tenant compte à la fois des variations environnementales et génotypiques pour évaluer le tallage.

Résultats

L'apparition des feuilles sur le brin maître et celle des talles à des rangs spécifiques sont des processus très coordonnées et robustes au travers des génotypes et des environements.

La principale différence génotypique réside sur la plus grande fréquence d'apparition des

rangs de talles précoces pour les hybrides à fort tallage, associée à des feuilles plus étroites et donc de plus petites surfaces (et donc demande en C).

Un indicateur de l'état trophique, estimant l'offre et la demande en assimilat de la plante par l'intermédiaire des caractéristiques morphogénétiques de la plante, explique la variation du nombre de talles maximum (TNmax) au travers des environnements et des génotypes. Néanmoins, les différences génotypiques dans la relation entre le S/D_{index} et le taux de tallage suggère qu'il existe un seuil de S/D_{index} différent entre variétés à fort et faible tallage : les variétés à fort tallage auraient une capacité à produire des talles précoces à partir d'un seuil de S/D_{index} plus faible. L'impact du niveau de compétition pour les assimlats C sur les fréquences d'apparition des premières talles est conforté par la corrélation négative entre la surface foliaire du brin maître et le potentiel de tallage.

Conclusions

Les différences génotypiques en terme de tallage sont associées (1) à des différences dans le niveau de compétition interne à la plante pour les assimilats C, reposant essentiellement sur la demande en C résultant des caractéristiques morphogénétiques des feuilles du brin maître (largeur) et (2) à un seuil différent de S/D_{index} permettant le tallage.

L'avantage d'un tel formalisme est sa simplicité d'application nécessitant des données expérimentales facilement accessibles. Son intégration dans des modèles dynamiques du peuplement végétal permettrait une prédiction beaucoup plus robuste en termes de croissance, utilisation des ressources (hydrique notamment) et rendement, par une meilleure prise en compte des mécanismes contrôlant la phase précoce de tallage.

Chapitre IV

Analyse génétique du tallage chez le sorgho sur la base d'un phénotypage assisté par modélisation

Introduction

Une meilleure compréhension des effets et de la fonction des QTLs controlant le tallage chez le sorgho peut améliorer l'efficacité de la sélection pour ce caractère d'intérêt pour les performances de cette culture, notammen dans des environnements semi arides. Le but de cette étude est de mettre en relation des QTLs de tallage avec des caractères phénotypiques élémentaires impliqués dans la régulation de ce caractère complexe, évalués par une démarche de phénotypage assisté par modélisation.

Matériel & méthodes

Huit populations BC2F2 de cartographie de sorgho, consistant de 50-100 individus, ont été suivi au champ à Gatton (SE du Queensland, Australie).

Phénotypage

Les caractères sélectionnés pour le phénotypage reposaient sur le cadre de modélisation dynamique dévelopé et présenté précédemment. Parmi les caractères ciblés, la dimension individuelle des feuilles du brin maître apparues au cours de la phase de tallage et la présence de talles à des stades et positions spécifiques ont été mesurées ; les observations phénotypiques on par ailleurs permis d'estimer les composantes génétiques du S/D_{index} pus de définir un seuil de S/D_{index} spécifique au tallage et génotype-dépendent.

Génotypage

Dans un premier temps, les huit lignées parentales BC1F4 des populations de cartographie (BC2F2) ont été intégrées parmi les lignées d'intérêt dans le cadre d'un programme de sélection pour la diversité de sorgho et génotypées par la technologie DArT^{TM}. Ce génotypage

préalable a permis d'identifier les régions d'introgression de *sorghum arundinaceum* dans le fond génétique du parent récurrent (*R39145-2-2*). Par la suite, un prélèvement du matériel végétal de tous les individus phénotypés pour chaque population a alors été réalisé pour leur génotypage sélectif à l'aide de marqueurs SSR.

Résultats

Trois QTLs associés avec le tallage ont été confirmés. Les QTLs sur le chromosome 3 (LG3) et 4 (LG4) affectent le tallage par l'intermédiaire d'un seuil de S/D_{index} pour le tallage affectant les rangs de talles précoces. De plus, le QTL sur LG4 semble avoir un effet sur la dimension des feuilles du brin maître en déterminant la largeur foliaire. Le QTL sur LG9, par contre, n'était pas associé avec la dimension foliaire ou le seuil de tallage et pourrait être relié à un processus non étudié. Sur cette base l'effet de telles QTL pourrait être incorporé dans le cadre de la modélisation du tallage selon les concepts du S/D_{index}.

Conclusions

Cette étude a permis d'identifier trois régions génomiques associées avec le tallage chez le sorgho et d'attribuer à chacune des fonctions élémentaires potentielles composant le contrôle du tallage par la compétition pour les assimilats carbonés. Ce travail illustre comment la modélisation peut assister, de façon pertinente dans le cadre de programme de sélection variétale, les études génétiques d'un caractère plastique et complexe.

Chapitre V

Modélisation du contrôle du tallage par la compétition dans la plante pour les assimilats carbonés : démonstration des concepts et simulation avec le modèle *EcoMeristem*

Introduction

Les modèles de simulation de la croissance des plantes et du peuplement ont été développés comme outil de simplification, d'intégration et de compréhension de la complexité biologique, puis d'aide à la décision. Ils se sont basés dans un premier temps sur des descriptions quantitatives du comportement dynamique de la culture ou de la plante, intégrant progressivement les processus biologiques élémentaires constitutifs de ce comportement global. Cependant, les processus contrôlant la morphogenèse de la plante et sa plasticité, non pas uniquement sur la base de l'offre environnementale mais aussi sur la base d'une demande interne (force de puits) propre à chaque génotype, n'ont que peu voire pas été intégrés dans de tels modèles. Dans ce contexte, le tallage des céréales est un processus morphogénétique clé à comprendre et formaliser dans ces modèles. Son étude récente et détaillée chez le sorgho, au travers d'une large gamme de conditions photo-thermiques et de génotypes, a permis de formaliser certains concepts clés de sa modélisation, a priori robustes.

Ce chapitre a pour objectif de confirmer, par des expérimentations complémentaires analysant la distribution et la disponibilité des sucres dans trois génotypes plus ou moins aptes au tallage et sous des conditions photothermiques contrastées, les concepts de modélisation du tallage récemment développé décrivant sa régulation par la compétition interne à la plante pour les assimilats carbonés. D'autre part, il s'agit de tester leur valeur ajoutée dans le cadre d'un modèle dynamique de croissance du peuplement et de la plante, *EcoMeristem*.

Matériel & méthodes

Les expérimentations réalisées au champ et en milieu controlé (chapitre II, III) ont permis de

développer les concepts de base pour expliquer le tallage comme conséquence de la compétition interne pour les assimilats carbonés, à travers notamment une équation estimant le rapport offre/demande (S/D_{index}).

Analyse de la distribution des sucres

Une expérience complémentaire a été réalisée pour explorer la distribution et la concentration des sucres dans les différents organes de trois hybrides représentatifs d'une variabilité génétique du tallage (LT, HT et Buster), et en comparant leur croissance (surface foliaire et biomasse) en condition plein soleil (S) et ombragée (Sh). Le stade pré-tallage, autour de la 4^{e} feuille ligulée sur le brin maître, a été choisi pour le dosage des sucres (glucose, fructose, saccharose et amidon) dans les différents organes de la plante (feuille 3 et 4 ligulées, reste des feuilles, gaines et racines).

Adaptation du modèle EcoMeristem pour le sorgho

Le modèle *EcoMeristem*, développé pour simuler et analyser la morphogenèse végétative du riz et sa plasticité phénotypique sous stress abiotique, a été adapté pour simuler la morphogénèse et le tallage chez le sorgho. Notamment, la distinction entre le phyllochron de l'apparition des feuilles (tip-phyllochron) et de leur ligulation (lig-phyllochron) a dû être implémenté, afin de mieux formaliser le cadre phénologique de synchronisation entre morphogénèse du brin maître et tallage. Par ailleurs, les concepts tels que déjà présents dans *EcoMeristem* pour dimensionner les feuilles en fonction du génotype (paramètre *MGR*, Meristem Growth Rate) et réguler le tallage en fonction d'un paramètre *Ict* de réponse au rapport Ic, offre/demande en assimilats carbonés, ont été conservés comme étant suffisamment proches des concepts de l'indicateur expérimental S/D_{index}.

Résultats

Le modèle *EcoMeristem* adapté a été calibré sur trois génotypes de sorgho dans un environnement donné puis validé dans un deuxième environnement. Les paramètres du

modèle résultants, MGR, Ict, SLAp (contrôlant l'épaisseur structurale des feuilles en fonction de leur rang) et Phyllo (tip-phyllochrone), ont permis de simuler de façon satisfaisante le comportement des différents génotypes (LT, 'low tillering', HT 'high tillering' et Buster, génotype fort tallage) dans deux environnements contrastés d'un point de vue photo-thermique. Un MGR élevé (i.e. fort accroissement de la taille des feuilles d'un rang de phytomère donné au suivant) et un Ict élevé (i.e, fort ratio Ic = S/D requis pour déclencher l'émergence d'une talle) ont été associés à l'hybride LT et un plus faible MGR et Ict à l'hybride HT et Buster. Ces caractéristiques génotypiques convergent avec les principaux résultats mis en exergue dans les analyses précédentes (chapitre II et III) utilisant l'équation S/D_{index} : MGR peut ainsi être rapproché des différences génotypiques mise en évidence dans la taille des feuilles (notamment leur largeur, estimée par le *leaf width increase rate*, LWIR) et Ict avec la valeur seuil de S/D_{index} pour le tallage. Par ailleurs une analyse de sensibilité menée avec le modèle Ecomeristem en faisant varier les paramètres MGR et Ict a confirmé la dualité existant au sein de la plante entre tallage et taille des feuilles. Un potentiel de tallage élevé s'est par ailleurs avéré associé de façon positive ou négative avec l'état des réserves carbonées, dépendant soit directement de la réponse constitutive du tallage aux sucres (paramètre Ict), soit du niveau de compétition entre les puits, ici défini par la dimension des feuilles (paramètre MGR). Les résultats des chapitres II et III tendent à être en faveur de la seconde hypothèse mais les optimisations des paramètres MGR et Ict au travers de trois génotypes représentatifs suggèrent que le dimensionnement des feuilles et le potentiel de tallage sont des caractères constitutifs qui interagissent pour déterminer le phénotype résultant : la dépendance (ou la dominance) d'un phénomène (tallage / taille des feuilles) par rapport à l'autre n'est pas totalement expliquée.

L'expérimentation complémentaire sur la distribution des sucres au stade de pré-tallage a montré une accumulation marquée mais transitoire de réserves en terme d'amidon dans les feuilles entre le matin et l'après-midi, expliquant notamment la variation diurnale de SLA et

semblant être relié à la capacité de tallage au travers des génotypes. Le traitement Sh a montré pour chacun des génotypes une réduction des teneurs en sucre globales dans la plante, ce qui confirme l'effet environnemental sur la disponibilité en assimilats carbonés et en conséquence sur le tallage. Cependant des différences génotypiques n'ont pu être relevées de façon claire, semblant indiquer des niveaux de sensibilités du tallage à la disponibilité en sucre dépendante du génotype.

Conclusions

Les résultats de cette étude ont permis de confirmer en grande partie la pertinence des concepts de modélisation précédemment développés, tant dans leur valeur physiologique (biologique) que dans leur capacité à améliorer la modélisation dynamique d'un peuplement de sorgho. Toutefois, la démonstration biochimique par les dosages de sucres des déterminants génotypiques du tallage reste à confirmer et autant que possible à affiner pour explorer la relation de cause à effet entre les caractère tallage et taille de feuilles. Par ailleurs, les concepts de modélisation ainsi validés doivent à court terme être utiliser pour améliorer la simulation du sorgho par le modèle dynamique agronomique APSIM.

PERSPECTIVES

Certains résultats de cette thèse restent à approfondir, en particulier: (i) les bases G et E de la relation entre le comportement métabolique (sucres) de la plants et sa capacité de tallage, (ii) la robustesse dans d'autres situations des QTLs de modèle détectés, (iii) l'amélioration du modèle *EcoMeristem* mais aussi APSIM pour leur connexion à de l'information génétique et définition d'idéotypes de sorgho.

Mises en relation d'information physiologique et génétique

- Les résultats préliminaires sur la distribution des sucres montrent de possibles corrélations entre le potentiel génotypique pour le tallage et sa réponse à l'état trophique (principalement l'amidon dans les feuilles et le saccharose dans les gaines). Cependant d'autres expérimentations sont nécessaires pour conforter et extrapoler ces résultats (investigation au cours de phase exponentielle de tallage et de sénescence des talles).
- L'analyse génétique du tallage a été réalisée sur un nombre limité de marqueurs génétiques (SSR) et d'autres régions en ségrégation, en particulier sur le chromosome 1 et 6, devraient aussi être explorées dans les différentes populations étudiées et donner d'autres opportunités pour détecter des QTLs reliés aux processus élémentaires du tallage. Par la suite ces QTLs pourraient être validés dans un autre fond génétique.
- Le séquençage complet du génome du sorgho procure de nouvelles opportunités pour les approches de comparaison génomique et de gènes candidats basés sur des gènes homologues en relation avec le tallage identifiés dans d'autres espèces
- Cette étude fournit de façon générale de solides preuves et les bases méthodologiques pour étudier et disséquer d'autres caractères morphogénétiques complexes d'intérêt agronomique.

Intégration de connaissances physiologiques dans les modèles

- Implémentation des concepts de modélisation dans deux modèles existants en cours
    - Dans *EcoMeristem*, d'autres adaptations pour améliorer la simulation du tallage sont en considération telles que l'introduction d'une notion de fenêtre temporelle d'opportunité d'émergence de talles en fonction de leur rang, et l'utilisation des concepts mis en évidence pour appuyer l'application du modèle à la phase reproductive (fertilité des talles et impact sur le rendement)
    - Dans APSIM, l'incorporation des concepts de modélisation est actuellement en

cours par le développement d'un module de tallage. Cela permettra de mieux simuler la plasticité du tallage et son impact dans la dynamique du peuplement végétal et donc de mieux évaluer ses effets sur le rendement dans un agro-écosystème donné, en particulier dans les environnements limités en eau.

- Modèles améliorés pour appuyer les analyses génétiques
    - En associant l'information génétique aux paramètres génétiques des modèles de 'nouvelles générations', comme cela a été fait pour le S/D_{index}, de nouvelles opportunités s'ouvrent pour appuyer les programmes de sélection en explorant '*in silico*' des génotypes virtuels en combinant différents allèles parmi les QTLs détectés et créer des idéotypes adaptés à des conditions environnementales spécifiques.
    - Dans le cas du tallage, il serait possible de sélectionner le comportement le plus adapté selon le système de pratique cultural*environnement considéré et acceptable pour les agriculteurs dans le contexte socio-économique

REMARQUES POUR CONCLURE

Cette thèse avait pour objectif la compréhension des déterminismes G et E de la régulation du tallage chez le sorgho par la compétition interne à la plante pour les assimilats carbonés .

Les principaux résultats de cette étude sont:

- La dynamique du tallage est un processus étroitement coordonné avec la morphogenèse du brin maître résultant en une compétition inhérente pour les ressources carbonées au sein de la plante pour la croissance des talles; une telle coordination est conservée au travers des environnements et des génotypes et

s'exprime notamment par l'effet de la compétition pour le C sur la fréquence d'apparition des talles précoces (où la compétition est la plus forte).

- Sur la base des résultats expérimentaux, un indicateur de compétition interne pour les assimilats carbonés (S/D_{index}) a été formalisé et suffisant pour expliquer la dynamique de tallage observée au travers de cinq environnements et six génotypes. Le S/D_{index} capture les déterminants G et E du développement de la surface foliaire, basé sur des mesures phénotypiques facilement réalisables, et tient compte à la fois de l'offre et de la demande en carbone au niveau de la plante.
- Les différences génotypiques dans la relation entre le S/D_{index} et le tallage montrent que les hybrides à fort potentiel de tallage ont une valeur seuil plus faible permettant la croissance des talles
- Les déterminismes E et G de la régulation du tallage par la disponibilité en C ont été confirmés en partie par l'analyse de la distribution des sucres dans la plante

Dans le cadre d'une étude génétique utilisant les composantes génotypiques du S/D_{index} , il a été possible :

- D'associer directement deux QTLs de tallage avec des processus élémentaires régulant le tallage et composant le S/D_{index}
- D'illustrer comment la modélisation de caractères complexes par dissection en processus élémentaires peut permettre d'appuyer les études génétiques de façon appropriée dans les programmes de sélection

Les premières essais pour améliorer le modèle *EcoMeristem* avec les concepts élaborés dans le cadre de cette étude ont montré la pertinence de la démarche et des premières simulations tenant compte des effets E et G.

"The Lord is my shepherd,
I shall not be in want.

He makes me lie down in green pasture,
he leads me beside quiet waters, he restores my soul.
He guides me in paths of righteousness for his name's sake.

Even though I walk through the valley of the shadow of death,
I will fear no evil, for you are with me;
your rod and your staff, they comfort me.

You prepare a table before me in the presence of my enemies.
You anoint my head with oil; my cup overflows.

Surely goodness and love will follow me all the days of my life,
and I will dwell in the house of the Lord forever. "

APPENDICES

➢ ORAL PRESENTATION

- 5th International Crop Science Congress (http://www.cropscience2008.com/)

13-18 Avril 2008

Jeju, SOUTH KOREA

UNRAVELLING COMPLEX ADAPTIVE TRAITS USING CROP MODELLING : EXAMPLE OF TILLERING IN SORGHUM

➢ POSTERS

- 5th Australian Sorghum Conference

30 Jan-2 Feb 2006

Gold Coast, AUSTRALIA

PHYSIOLOGY & GENETICS OF TILLERING IN SORGHUM

- Gene-Plant-Crop relations Congress

23-26 April 2006

Wageningen, THE NETHERLANDS)

MODEL ASSISTED ANALYSIS OF TILLERING RESPONSE TO ENVIRONMENT OF 6 SORGHUM GENOTYPES

5th International Crop Science Congress, Jeju in Korea
April 13~18, 2008, ICC Jeju, Korea

Unravelling complex adaptive traits using crop modelling: physiology and genetics of tillering in sorghum

Hae-Koo Kim [1,2], Delphine Luquet [1], Graeme Hammer [2], Erik van Oosterom [2], Michael Dingkuhn [1]

[1] CIRAD, UPR 59, Avenue Agropolis Lavalette TA A 59/01 34398 Montpellier, France

[2] The University of Queensland, School of Land, Crop and Food Sciences, Brisbane QLD 4072, Australia

Corresponding author: haekookim@yahoo.com

Abstract

Recent advances in functional genomics generated a huge amount of genetic information of high value for crop improvement. However, DNA sequences alone have little value in unravelling the genetic and physiological bases of complex traits controlled by a large number of genes. This emphasises the importance of approaches combining genetics with physiology, to dissect complex traits into basic biological processes less prone to environmental variations and related to a smaller number of genes. In this context, modelling is essential to identify and quantify such processes and their interactions with environment resulting in complex trait expression. This work aimed to show the added value of formalizing physiological knowledge in models to analyse the genetic control of sorghum tillering, a complex agronomic trait of interest. Detailed physiological analyses were previously realised to implement and test modelling concepts in which tillering is controlled by plant internal competition for carbon assimilates, depending on genotypic characteristics (demand for large vs. many organs) and environment (resource availability). These concepts were used to analyse experimental data acquired on eight BC2F2 populations of sorghum, derived from a cross between *Sorghum arundinaceum* and an elite tester. This analysis largely confirmed the modelling concepts previously developed and allowed to test the link between three putative QTLs and model based coefficients. The implications of this approach for reducing QTL*E effects in QTL detection and potential for future model assisted genetic analyses are discussed. This study illustrates how modelling can assist genetic studies on complex traits and assist breeding programs.

Media summary

Integrating knowledge gained in crop physiology through crop modelling can support the genetic analysis of complex traits, thereby enhancing breeding efficiency.

Key words

Plant internal competition, carbohydrate supply/demand ratio, QTL detection

MODELISATION DU CONTROLE ENVIRONMENTAL ET GENETIQUE DU TALLAGE CHEZ LE SORGHO

RESUME

Cette thèse développe un cadre conceptuel de modélisation formalisant les composantes environmentales (E) et génétiques (G) du contrôle du tallage par la disponibilité en assimilat carboné (C) chez le sorgho (rapport offre/demande, S/D). Ce concept a été élaboré et testé au travers de cinq expérimentations et six génotypes aux capacités de tallage différentes. Les résultats ont démontré que la régulation du tallage est étroitement liée à sa compétition avec le développement et la morphogenèse des feuilles du brin maître, affectant notamment la fréquence d'apparition des talles précoces. Un indicateur de compétition en C dans la plante, S/D_{index}, permettant d'expliquer le tallage au travers de la gamme étudiée de G et E, a ainsi pu être développé. Une fois confirmé par l'analyse de la distribution des sucres dans la plante, cet indicateur a été appliqué (i) pour appuyer une étude génétique ayant permis d'identifier trois QTLs associés au tallage, dont deux spécifiquement associés avec des composantes génétiques du S/D_{index} (relatives à la largeur des feuilles et à un seuil S/D de tallage) ; (ii) initier l'amélioration d'un modèle existant (*EcoMeristem*). Certains résultats de ce travail restent à approfondir: (i) les bases G et E de la relation entre la dynamique des sucres dans la plantes et la capacité de tallage, (ii) la robustesse dans d'autres situations des QTLs de modèle détectés, (iii) l'amélioration du modèle *Ecomeristem* mais aussi APSIM pour leur connexion à de l'information génétique et définition d'idéotypes de sorgho.

MODELLING ENVIRONMENTAL AND GENETIC CONTROL OF TILLERING IN SORGHUM

ABSTRACT

This thesis develops a conceptual modelling framework formalizing the environmental (E) and genetic (G) components of tillering control by carbohydrate (C) assimilate availability in sorghum (supply/demand ratio, S/D). This concept was elaborated and tested across five experiments and six contrasting genotypes in terms of tillering ability. The results showed that regulation of tillering was strongly related to its competition with main stem development and leaf morphogenesis, by influencing the appearance frequency of the lower-rank tillers. An indicator of internal competition for C, S/D_{index}, was developed and allowed to explain tillering response across the range of G and E investigated. Once confirmed by analysis of sugar distribution within the plant, this indicator was applied (i) to support a genetic study, which identified three quantitative trait loci (QTLs) associated with tillering ability, two of which could be specifically associated with genetic components of S/D_{index} (related to leaf width and a S/D threshold for tillering); and (ii) to improve and evaluate an existing plant model (*EcoMeristem*). Results of this study open new opportunities to investigate the following: (i) G and E bases of the relationship between C dynamics and tillering ability, (ii) the stability of model-based QTLs and (iii) further improvement of *EcoMeristem* and other models such as APSIM to connect them to genetic information and help develop new sorghum ideotypes.

MOTS-CLES: *Sorghum bicolor* (L.) Moench, compétition interne, rapport offre-demande, état trophique, dissection écophysiologique, dévelopment de la surface foliaire, largeur foliaire, seuil de tallage, cadre de modélisation, QTL

KEYWORDS: *Sorghum bicolor* (L.) Moench, internal competition for carbohydrate, supply-demand ratio, ecophysiological trait dissection, leaf area development, tiller hierarchy, leaf width, tillering threshold, modelling framework, QTL

INTITULE ET ADRESSE DES LABORATOIRES D'ACCUEIL

UPR59 "Integrative Modelling : Phenotypic Plasticity & Crop performances", CIRAD, Avenue Agropolis Lavalette TA A 59/01 34398 Montpellier Cedex 5, FRANCE

APSRU, The University of Queensland, School of Land, Crop and Food Sciences, QLD 4072, Brisbane, AUSTRALIA